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Biomedical Applications of Magnetic Fluids. II. Preparation and Magnetic Guidance of Magnetic Albumin Microsphere for Site Specific Drug Delivery in Vivo

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Biomedical Applications of Magnetic Fluids. II.

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Magnetic Guidance of Albumin Microsphere

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# BIOMEDICAL APPLICATIONS OF MAGNETIC FLUIDS. II.[1] PREPARATION AND MAGNETIC GUIDANCE OF MAGNETIC ALBUMIN MICROSPHERE FOR SITE SPECIFIC DRUG DELIVERY IN VIVO*

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* This work was presented at the 100th Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, April 1980.

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Magnetic guidance of magnetic albumin microsphere for site specific delivery was investigated in mice and rats. After intravenous injection in mice, magnetic microspheres with 1 and 3 μm diameter size were localized and retained in the target-site (lung) by application of two permanent magnets to the lungs. Injection into the renal artery in rats also indicated that the 1-μm microspheres were concentrated in the kidney by a magnetic field. When the magnets were not applied, however, the microspheres following intravascular injection were concentrated mainly in the liver, regardless of the route of administration. Such preferential localization by magnetic means suggested that magnetic albumin microspheres could become effective drug carriers with site specificity for the delivery of chemotherapeutic agents in cancer therapy.

Keywords——magnetic albumin microsphere; magnetic fluid; drug carrier; site specificity; drug delivery; magnetic guidance; tissue distribution

One of the goals in cancer chemotherapy has been to find means for directing drugs selectively to tumor sites. The most interesting approach to this problem today is to entrap potent drugs in some drug carriers which are non-toxic and biodegradable. Various investigators have attempted to develop useful drug carriers, based on evaluation *in vitro* and *in vivo* experiments.[2,3] Recent examples include antitumor agents into liposomes,[4] albumin microspheres,[5] and microspheres in oil emulsion.[6] We recently reported that 5-fluorouracil entrapped in albumin microspheres was present in high levels in the liver of mice after intravenous injection,[7,8] and suggested sustained release and prolonged action of entrapped drug occurred in Ehrlich ascites[9] and solid carcinoma.[10] More recently, we reported that adriamycin entrapped in albumin microspheres showed pronounced antitumor activity on AH 7974 liver metastasis in rats as a model with an experimental tumor.[11] However, intravenous injection of those drug carriers as mentioned above results in their uptake predominantly by the reticuloendothelial system,[7,8,12] especially the Kupffer cells in the liver.[13]

Recently, Widder *et al.*[14] suggested that since magnetic microspheres injected into the ventral caudal artery could be localized to some extent to a predetermined tail segment by an externally applied magnetic field, magnetically guided albumin microspheres entrapped in drugs would be useful as a drug delivery system with site specificity. Kato *et al.*[15] also reported the possibility of magnetic control of antitumor drug and preparation of the ferromagnetic mitomycin C

microcapsules with sustained release property, responding to necessity of selective cancer chemotherapy.[15] If site specific drug delivery of antitumor agents could be achieved with magnetic means, this delivery system would eliminate adverse side effects that are often the sequelae of generated systemic drug distribution. This paper describe the utility of the magnetic albumin microsphere (ferro-colloid-entrapped albumin microsphere) as a drug carrier with target specificity by measuring microsphere levels in the lung and kidney after intravenous and intra-renal-arterial administration, respectively. A short communication of this work has been published.[1]

MATERIALS AND METHODS

Materials——Bovine serum albumin (BSA), fraction V powder (Seikagaku Kogyo Co. Ltd.) and $^{125}$I-human serum albumin ($^{125}$I-HSA, Japan Radioisotope Association) were used. A part of water-based magnetic fluid which was a suspension containing $Fe_3O_4$ fine particles, that is magnetites, was obtained from Taiho Industries Co. Ltd. and colloidal magnetite was prepared by the method of Shimoiizaka *et al.*[16] Permanent magnet (Super Disc Magnet, No. 30730, inner radius 5 mm, outer radius 9.5 mm, thickness 6 mm, Edmund Scientific) with a magnetic introduction of about 3000 Gauss was used.

Animals——Male mice of ICR strain weighing about 30 g and male rats of Wistar strain weighing 210-250 g were used. The animals were maintained in a environment of controlled temperature at 24 ± 1° and provided with Oriental regular solid diet and tap water *ad libitum*.

Preparation of Magnetic Albumin Microsphere——Magnetic albumin microspheres were prepared with BSA, $^{125}$I-HSA, and magnetic fluid by a modification of the method of Widder *et al.*[17] Two hundred mg of BSA labeled with 2 mg $^{125}$I-HSA and 0.5 ml magnetic fluid were added to 2 ml distilled water. This was added to 200 ml of 10 %(v/v) Span 85 in cottonseed oil and the mixture was emulsified with Ultra Turrax® (Ika Werk) at 8000 or 20000 rpm for 30 min at 10°. Heat hardening of microspheres was accomplished by exposure to temperature of 180° for 10 min. Since the range of particle

size of microspheres prepared at 8000 rpm was 1-7 μm, the 1-2 and 5-7 μm microspheres were separated from the 2-4 μm microspheres by centrifugation of the microsphere suspension. When the range of particle size of microsphere prepared at 20000 rpm was 0.4-1.5 μm, the microspheres were used without further separation. The mean particle size of microspheres was determined by a photomicrographic method. The magnetic microspheres are shown in the scanning electron microsphere photomicrograph in Figure 1a and the presence of magnetites on the surface of microspheres was observed with the energy dispersive-type X-ray microanalysis (Figure 1b). The final microsphere contain about 50 % magnetites by dry weight.

Fig. 1

In Vivo Model for Carrier Targeting — In the first experiments, the mouse lung was selected as a model for in vivo testing of microspheres for two reasons: (i) The lung tumor occupies high ratios in many malignancies, and (ii) the microspheres after intravenous injection pass through the lung until sequestration in the liver. Each mouse was fixed on its back without anesthesia. One mg of the 1 or 3 μm microsphere was injected into the tail vein in mice in 0.2 ml of a suspension. Two magnets were directly applied to the side of each mouse, that is, the breast and back of mice, throughout the experiment so as to concentrate the microspheres into the lung. The mice were killed 10 or 60 min after administration,

and $^{125}$I-labeled microspheres in various tissues were determined by an auto-gamma scintillation spectrometer (Type 5110, Pakard).

The kidney was selected as a model in the second experiments. It is difficult to collect the microspheres in other tissues except the lungs or heart after intravenous injection, so the route of administration was changed to intra-arterial injection. Each rat was anesthesized with pentobarbital sodium at a dose of 60 mg/kg i.p., and was fixed on its back. Administration of the microspheres through the renal artery was carried out by cannulating a polyethlene tubing and forming a new vessel bypassing the original artery, because a polyethylene tubing is easy to be used for an injection. One mg of the 1 μm microsphere was injected into the left renal artery. Two magnets were directly placed throughout the experiments at both sides of the left kidney which was exposed by a midline abdominal incision. Distribution of microspheres 10 and 60 min after administration was examined by the method described previously. The distribution of $^{125}$I-albumin microspheres to various organs is represented as % of dose per g tissue or that in the whole tissue.

RESULTS

Magnetic Responsiveness of Magnetic Microspheres

The magnetic responsiveness of the microspheres suspended in 0.2 %(v/v) polysorbate 80 solution is shown by the sequential time sequence photographs in Figure 2. It is obvious that the microspheres were sequentially localized on and around the disc-magnet. In that follow, it was tested in detail whether or not the microspheres were preferentially relocated in target sites by a magnetic field applied when the microspheres with highly magnetic responsiveness were intravenously injected to animals.

Fig. 2

Tissue Distribution of Microspheres in Mice

Tissue distribution of radioactivity at 10 min after intravenous injection of magnetic microspheres is shown in Figure 3. After injection of the 1 μm microspheres in mice without magnet (control), about 3.9 % of the administered dose (15.8 %/g tissue at the concentration) was found in the lung. When the particle size of the microspheres was enlarged from 1 to 3 μm in diameter, uptake of the 3 μm microsphere in the lungs increased from 3.9 % of dose (15.8 %/g tissue) to 10.7 % (51.7 %/g). When two magnets were applied to the lungs, the microsphere levels in the lungs increased about four-fold for the 1 μm microspheres and twice for the 3 μm microsphere compared with each control. After injection of the 3 μm microspheres.

a peak lung level (104.0 %/g tissue) was measured, but the amount of the microspheres in the lungs was only 21.6 % of the dose.

Fig. 3

When the 3 um microspheres were injected in mice, tissue distribution of radioactivity at 60 min is as shown in Figure 4, where there is also the distribution when two magnets were applied to the lungs only for the initial 10 min. The uptake of the microspheres in the lungs increased from 17.0 % of the dose (67.2 %/g tissue) to 28.2 % (111.3 %/g) and that in the spleen and liver decreased when two magnets were applied for 60 min. With application of two magnets for the first 10 min, there was an initial distribution of radioactivity to the lungs (Figure 3) followed by rapid clearance from the lungs and localization in the liver (Figure 4).

Fig. 4

Tissue Distribution of Microspheres in Rats

Figure 5 compares the tissue distribution of the 1-μm microspheres at 10 min following intravenous and intra-renal-arterial administration with or without application of two magnets to the left kidney. After intravenous injection, the microspheres did not concentrate in the kidney and localized mainly in the liver regardless of application of two magnets to the kidney. The microsphere

level in the kidney after intraarterial administration was higher than that after intravenous injection with and without magnets. Administration into the rat renal artery with magnets, on the other hand, concentrated the microspheres on the kidney at 10 min to 56.4 % of the dose (47.7 %/g tissue), and the value was about 2.5-fold higher than that in the control.

Fig. 5

Figure 6 compares the tissue distribution of the 1 μm microspheres at 60 min following intra-renal-arterial administration with application of two magnets to the left kidney for the initial 10 or 60 min. Microsphere level in the left kidney was found to be 56.4 % of the dose (47.7 %/g tissue) at 10 min after administration, when applying the two magnets.[1] In contrast to this value, the fraction of the dose remaining at the same organ at 60 min was much less when the magnet was removed 10 min after administration.

Fig. 6

DISCUSSION

Recently, in several labolatories, it has come to be realized that magentic microspheres with magnetic responsiveness may be used as target-selective homing devices in cancer chemotherapy.[14,15,17] Also, a considerable amount of infomation was presented regarding the fate of the microspheres and their contents after injection to rats.[14,21] It is, however, experimentally obscure whether magentic guidance of the microspheres to the target sites such as the lung and kidney is possible. In addition, no infomation has been amassed regarding the effect of application time of the magnets to the target sites and removal from the sites on the behavior of the microspheres in blood. From a preliminary report[1] and these studies, it is evident that if the administration route of the microspheres is carefully selected and application time of magnets to the target sites is extended magnetic guidance of the microspheres to the sites such as the lung and kidney and the retention of the microspheres in those sites is possible (Figures 5 and 6).

A small fraction of the intravenously administered dose distributed to the lungs is due to a simple filter effect of the pulmonary capillary beds, although vascular constriction may play some role in the trapping process. A large fraction is distributed mainly in the liver high in reticuloendothelial cell activity. This phenomenon of filtration in the lungs was clearly observed when the particle size of the microspheres changed from 1 μm in diameter to 3 μm.

However, with the 1 or 3 μm microspheres injected in mice, there was a large distribution to the lungs by the application of the magnets, compared to no application of the magnets (Figure 3). Moreover, distribution of the microspheres to the kidneys following the intra-renal-arterial administration due to a simple filtration of the superficial and juxtamedullary glomerular layers[18] was not negligibly but the microsphereleve1 in the kidney with the magnets was higher than that without the magnets (Figure 5).[1]

With the 3 μm microspheres injected in mice, retention in the lungs when applying the magnets was greater than that without application.[1] Those microsphere levels retained by an externally applied magnetic field in the lungs clearly decreased when the magnets were removed (Figure 4). The pattern of distribution in the kidney when the magnets were removed was similar to that in the lung (Figure 6). The decrease in the lungs or kidney seen between 10 and 60 min following administration of the microspheres may be due to washout within the lungs or kidney so that the microspheres initially trapped are allowed to recirculate in the bloodstream. The results obtained from the experiments of removing the magnets indicate that the application time of the magents to the target sites is important for retention of microspheres in those sites.

A guarded selection of the administration route is also important for transporting microspheres to target sites. Kanke et al.[19] reported that no clearcut difference in distribution patterns was observed between intravenous and intraarterial administration of microspheres. Also Sjöholm et al.[20] reported that the route of administration had no effect on the gross distribution pattern by studying the distribution of the polyacrylamide microparticles after intravenous and intraperitoneal injection in mice. The results shown in this paper, however, found a clearcut difference in the distribution pattern to be present between intravenous and intraarterial administration of microspheres in rats. Thus, the distribution of the microspheres *in vivo* after different routes of administration is really complicated, and further experiments and sufficient discussion would be needed.

The amount of microspheres retained at the target site (lung in mice) was 28.2 % of the dose at 60 min after intravenous injection of the 3-μm microspheres at a field strength of about 3000 Gauss. Failure to achieve greater retention of microspheres is most probably due to weak magnetic strength. When the strength of magnetic field is increased, this value might be improved considering that Widder et al.[14,21] used high magnetic field strength (8000 Oe) to retain 37-65 % microspheres into the tail after infusion through the ventral caudal artery. As mentioned above, many drug carriers which are capable of transporting drug molecules from the site of application

directly to the site of action have been developed.[2,3] In the near future some carriers of them may be used in the clinical field, but the utility of some drug carriers is restricted because of the lack of specificity or selectivity. Controlled localization of drug carriers, however, has been difficult to achieve. Recently, Gregoriadis[22] documented that attempts have been made to rationalize liposome development by tailoring their structure to the particular biological milieu in which they are intended to act. In the case of magnetic albumin microspheres,it is certain that this drug carrier can be used as a target-selective homing device.

Studies are presently under way in our laboratory to examine the site specificity of the drug and its antitumor effect against experimental lung tumor after intravenous injection of magnetic albumin microsphere-entrapped antitumor agents.

Acknowlegement The authors are grateful to the Taiho Industries Co., Ltd. for a supply of water-based magnetic fluid.

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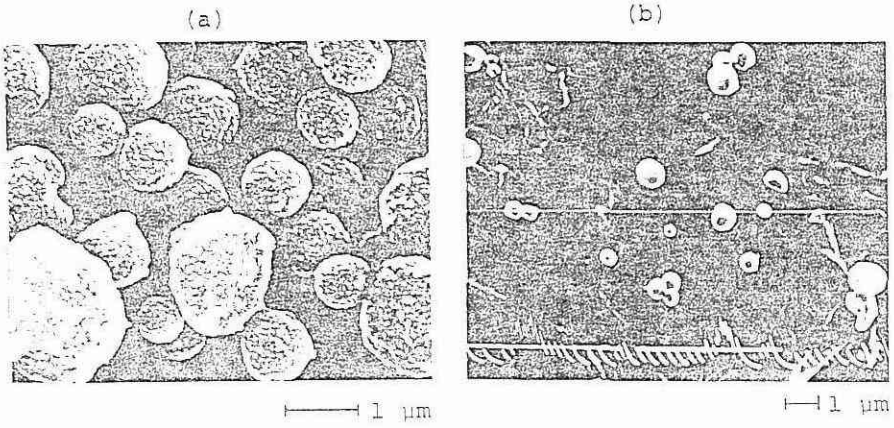
Fig. 1



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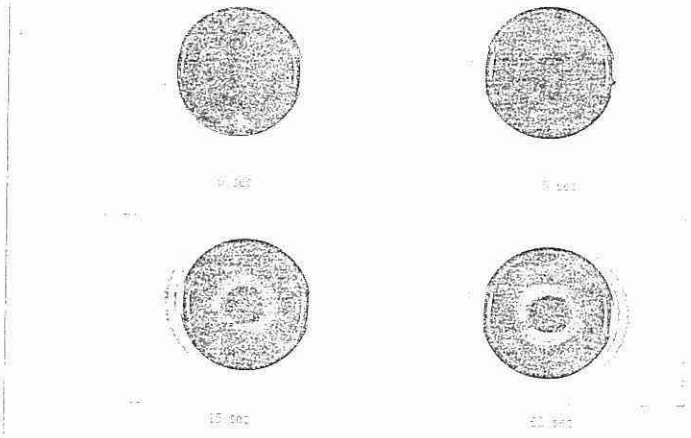

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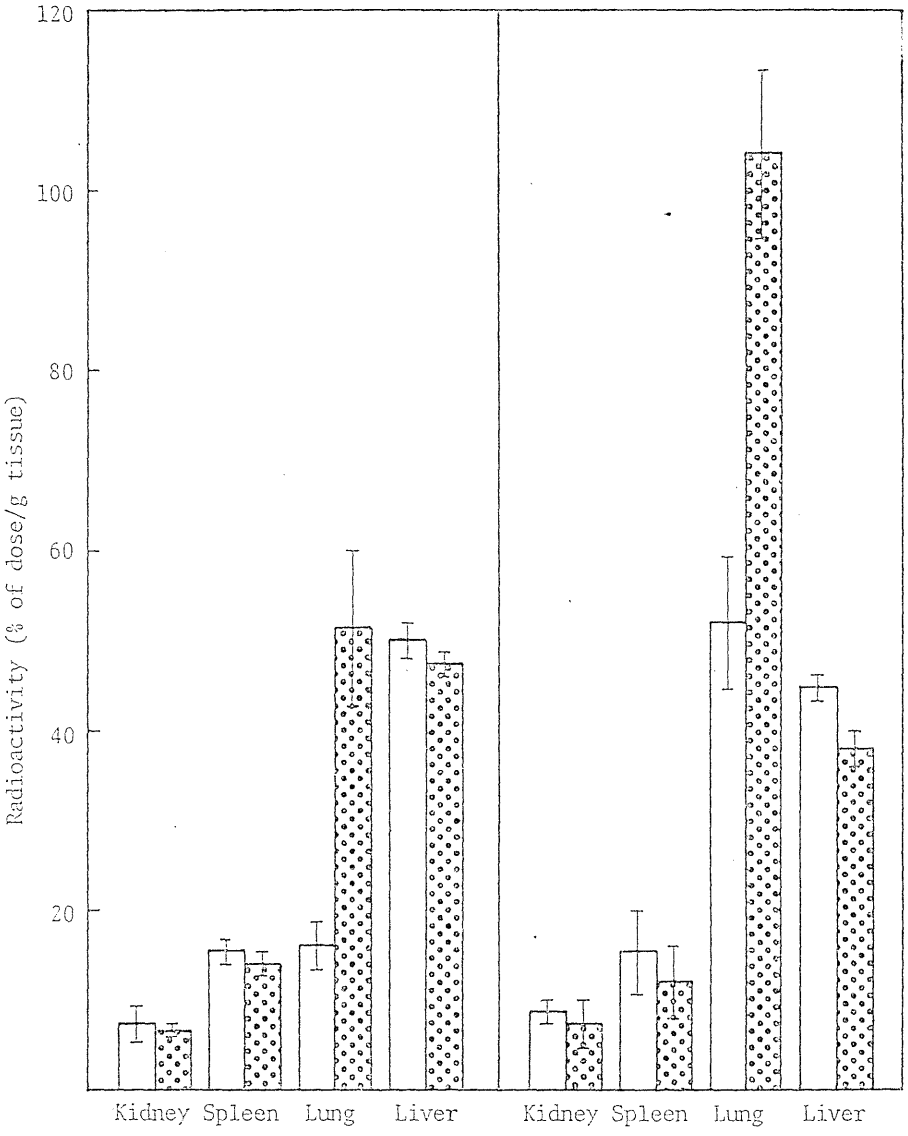
120
100
80
60
40
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Radioactivity (% of dose/g tissue)
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Spleen
Lung
Liver
Kidney
Spleen
Lung
Liver

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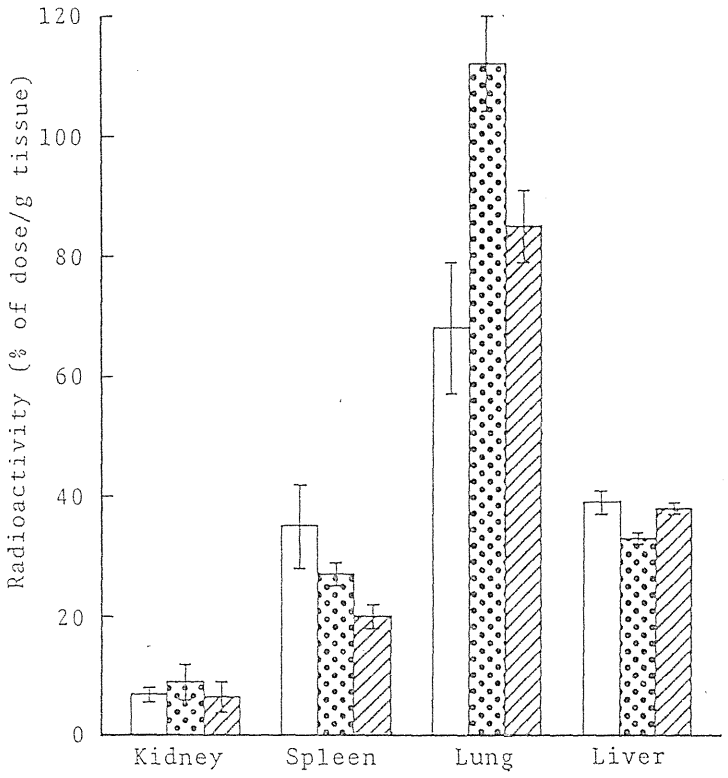
Fig. 4.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100
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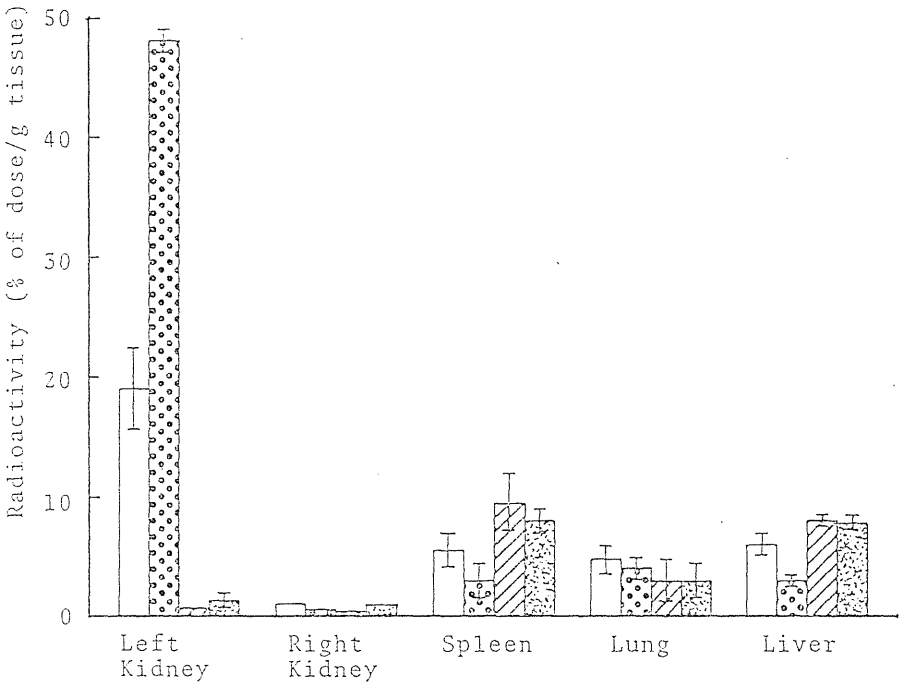
Kidney
Spleen
Lung
Liver

Fig. 5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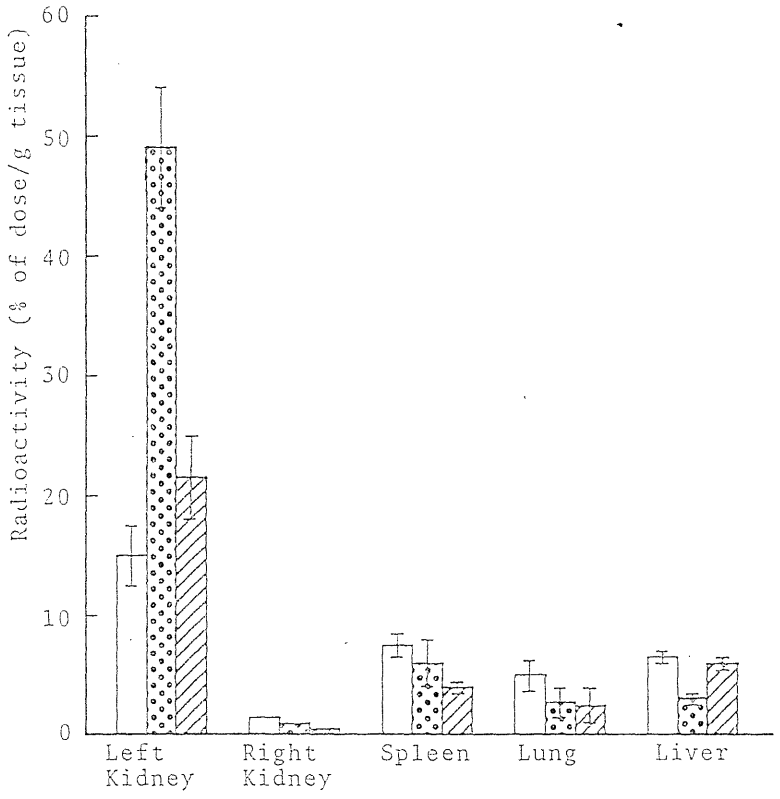
Right Kidney
Spleen
Lung
Liver

Fig. 6.


Radioactivity (% of dose/g tissue)
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Left Kidney
Right Kidney
Spleen
Lung
Liver

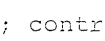
FIGURE CAPTIONS

Fig. 1. Scanning Electron Micrograph of Magnetic Microspheres and Spectrogram Obtained with an Energy Dispersive-type X-ray Microanalysis

Fig. 2. Sequential Time Sequence Photographs of Magnetic Microspheres in Aqueous Suspension after a Disc Magnet is applied

Fig. 3. Tissue Distribution of Radioactivity at 10 min after Intravenous Injection of Magnetic Microspheres in Mice (1 and 3 μm in diameter)

A; 1 μm in average diameter, B; 3 μm in average diameter

 ; control (no magnet),  ; treatment with two magnets throughout the experiments.

Results are expressed as the mean ± S.E. of 3-5 mice.

Fig. 4. Tissue Distribution of Radioactivity at 60 min after Intravenous Injection of the 3-μm Magnetic Microsphere in Mice

 ; control (no magnet),  ; treatment with two magnets for 60 min,  ; treatment with two magnets for the initial 10 min.

Results are expressed as the mean ± S.E. of 3-5 mice.

Fig. 5. Tissue Distribution of Radioactivity at 10 min after Intravenous or Intraarterial Administration of the 1-μm Microspheres in Rats

 ; intra-renal-artery (no magnet),

 ; intra-renal-artery (with magnet),

 ; intratail vein (no magnet),

 ; intratail vein (with magnet)

Results are expressed as the mean ± S.E. of 3-5 rats.

Fig. 6. Tissue Distribution of Radioactivity at 60 min after Intra-renal-arterial Administration of 1-μm Magnetic Microspheres in Rats

 ; control (no magnet),  ; treatment with two magnets for 60 min,  ; treatment with two magnets for the initial 10 min.

Results are expressed as the mean ± S.E. of 3-5 rats.

# 論　文　目　録

主　論　文

1. 題　名　薬物担体としてのアルブミン小球体に関する研究

2. 印刷公表の方法及び時期



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第4章　AH 7974転移肝癌に対するadriamycin含有小球体の抗腫瘍効果

第1節　緒　　言

第2節　実験材料及び方法

第3節　実 験 結 果

第4節　小括と考察

Chem. Pharm. Bull.(Tokyo), **29**, (5) 1315～1321 (1981)

第5章　磁性アルブミン小球体による臓器選択性の増強

第1節　緒　　言

第2節　実験材料及び方法

第3節　実 験 結 果

第4節　小括と考察

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J. Pharm. Dyn., **4**, (8) 624～631 (1981)

Chem. Pharm. Bull. 27(1) 204—209 (1979)

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# Drug-carrier Property of Albumin Microspheres in Chemotherapy. II.[1] Preparation and Tissue Distribution in Mice of Microsphere-entrapped 5-Fluorouracil[2]

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Bovine serum albumin microspheres containing 5-fluorouracil-6-$^3$H were prepared by heating at 180° (or 150°, 100°) of 25% albumin solution in cottonseed oil emulsion. The shape of this microsphere was invariably spherical, and the average diameter was 0.66 $\mu$. After intravenous injection in mice, 5-fluorouracil-6-$^3$H entrapped in albumin microspheres localized mainly in the liver, and the disappearance rate of radioactivity in microspheres from the tissue was very slow in comparison with that of free drugs. The microsphere might be delivered into reticuloendothelial system in the liver because of its phagocytic activity, as well as the distribution following injection of albumin macroaggregates. Such preferential localization and sustained release of entrapped drugs suggested that albumin microspheres are useful as drug-carrier in chemotherapy.

**Keywords**——albumin microsphere; antitumor agent; drug-carrier; reticuloendothelial system; phagocytosis; drug distribution

After the drug is absorbed or injected into the blood stream, it may be distributed generally throughout the body in the medium of the body fluids. It is well known that the rate, extent, and pattern of the distribution are determined by the physicochemical characteristics of the drug. As antitumor agents frequently exert undesirable toxic effects, localization of the drugs to tumor sites would reduce the side effects. Then it is desirable that the antitumor drug selectively reaches its target tissues in drug therapy.

Past approaches to targeting of chemotherapeutic agents have been largely related to several drug carriers. Most tumor cells have been shown to phagocytize some spheres.[4,5] If a drug carrier containing an antitumor agent is phagocytized into tumor cell, the carrier may be digested with lysosomal enzymes in the cell and free active drug may be then released to the environments.

Trouet, *et al.*[6] reported reduced toxicity and increased effectiveness in the treatment of leukemia when DNA complexes of antitumor agent daunomycin were pinocytized from solution. Furthermore, they found that the free active drug could be released from DNA complex after digestion of the complex with lysosomal enzymes. The use of liposomes (phospholipid vesicles) as drug carriers has been noted recently. Gregoriadis and his coworkers[7] reported that liposome-entrapped drugs intravenously injected into rats were concentrated in the liver and the spleen by phagocytosis in the reticuloendothelial systems. Gregoriadis and Neerunjun[8] investigated the possibility of homing liposomes to target cells by using liposomes associated with molecular probes which exhibit a specific affinity for the surface of a variety of normal and malignant cells. Radiologists utilized the phagocytic activity of the liver and the spleen to study and diagnose the function of reticuloendothelial system by radiolabeled albumin microspheres.[9] Kramer, *et al.*[10] reported that human serum albumin microspheres containing 6-mercaptopurine were phagocytized by HeLa and glioblastoma *in vitro*, and they suggested the possibility that the albumin microspheres could be utilized as drug carrier.

We have recently reported that 5-fluorouracil (5-FU) entrapped in bovine serum albumin microspheres localized in the liver after intravenous injection in mice.[1] In this paper we wish to report physicochemical properties of albumin microsphere and its usefulness as a drug carrier.



## Experimental

**Materials**——5-Fluorouracil-6-$^3$H ($^3$H-5-FU) was purchased from Japan Radioisotope Association (Tokyo, Japan). Unlabeled 5-FU was supplied from Kyowa Hakko Co., Ltd. Bovine serum albumin was obtained from Seikagaku Kogyo Co., Ltd. and cottonseed oil was selected as a vegetable oil.

**Preparation of Albumin Microspheres**——Bovine serum albumin microspheres containing antitumor agent $^3$H-5-FU were prepared as follows by modification of the method of Scheffel, *et al.*[9b] (Chart 1). Two hundreds and fifty mg of bovine serum albumin were dissolved in 1 ml of radiolabeled 5-FU solution. The resulting solution was mixed with 100 ml of 10% span 85 in cottonseed oil, and homogenated with a motor driven glass stirrer (Tokyo Rikakiki, Model MS-75) at about 2500 rpm for 10 min, and was furthermore emulsified with an ultrasonic homogenizer (Nihonseiki Seisakusho, Model G50022-4) at 100 W for 30 min.

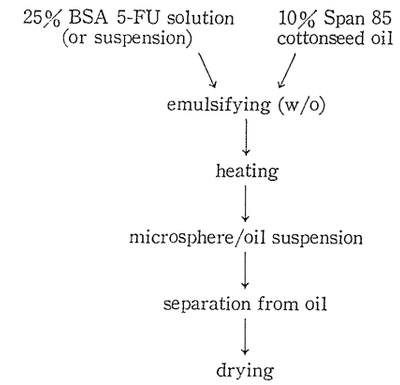


Chart 1. Schematic Diagram of Preparation of Albumin Microsphere Containing 5-Fluorouracil

Another 100 ml of cottonseed oil were heated to 100°, 150°, or 180° in a 500 ml three necked round-bottomed flask under continuous stirring by a glass stirrer at 2500 rpm. The homogenated albumin-oil was gradually added to the heated oil, the temperature was adjusted to 100°, 150°, or 180°, and heating and stirring was maintained for 10 min. After the suspension was cooled to room temperature, each suspension was mixed with 200 ml of diethyl ether. The mixture was separated by centrifugation and the oil–ether phase was discarded. For complete removal of adhering oil, the preparate was washed in ether and in ethyl alcohol. After washing the spheres, the precipitates were stored in a desiccator. And just before experiments, the dried microsphere was dispersed with 0.2% polysorbate 80 and sonicated at 100 W for 10 min so as to remove the free drug loosely adhered to the surface of the microspheres. After centrifugation, the precipitate was dispersed with 0.2% polysorbate 80 in 0.9% NaCl solution and suspended well with an ultrasonicator.

**Preparation of Albumin Macroaggregates**——Bovine serum albumin macroaggregates containing the $^3$H-5-FU were prepared by a slight modification of the method of Thomas, *et al.*[11] The macroaggregates were prepared on demand. Twenty five mg of bovine serum albumin were dissolved in 1 ml of radiolabeled 5-FU solution. The pH of the solution was adjusted to 5.7±0.2 with 0.1 N HCl. And the solution was heated at 90° for 10 min under continuous stirring. In order to wash the macroaggregates and to remove

1) Preceding paper, Part I: K. Sugibayashi, Y. Morimoto, T. Nadai, and Y. Kato, *Chem. Pharm. Bull.* (Tokyo), **25**, 3433 (1977).
2) Part of this work was presented at 98th Annual Meeting of Pharmaceutical Society of Japan, Okayama, April, 1978.
3) Location: *a) 1-1 Keyakidai, Sakado, Saitama, 350-02, Japan*; *b) Kita 12-jo, Nishi 6-chome, Kita-ku, Sapporo, 060, Japan.*
4) R.F. Gilfillian, *Cancer Res.*, **28**, 137 (1968).
5) I. Palyi, *Arch. Geswulstforsch*, **33**, 345 (1969).
6) *a)* A. Trouet, D.D. Campeneere, and C. de Duve, *Nature* (London), **239**, 110 (1972); *b)* A. Trouet, D.D. Campeneere, M.D. Smedt-Malengreaux, and G. Atassi, *Europ. J. Cancer*, **10**, 405 (1974).
7) *a)* G. Gregoriadis and B.E. Ryman, *Europ. J. Biochem.*, **24**, 485 (1972); *b)* G. Gregoriadis, *FEBS Letters*, **36**, 292 (1973).
8) G. Gregoriadis and E.D. Neerunjun, *Biochem. Biophys. Res. Commun.*, **65**, 537 (1975).
9) *a)* I. Zolle, B.A. Rhodes, and H.N. Wagner, Jr., *Int. J. Appl. Radiat. Isotop.*, **21**, 155 (1970); *b)* U. Scheffel, B.A. Rhodes, T.K. Natarajan, and H.N. Wagner, Jr., *J. Nucl. Med.*, **13**, 498 (1972).
10) *a)* P.A. Kramer, *J. Pharm. Sci.*, **63**, 1646 (1974); *b)* P.A. Kramer and T. Burnstein, *Life Sci.*, **19**, 515 (1976).
11) J. Thomas and S.N. Wiener, *Int. J. Appl. Radiat. Isotop.*, **25**, 463 (1974).

free 5-FU, small amount of water was added to this macroaggregates and the suspension was sonicated at 100 W for 10 min. After centrifugation, 10 ml of 0.9% NaCl solution was added to precipitate. And the suspension was obtained by dispersing the mixture with an ultrasonic cleaner (Branson, Model 220) and used for animal experiments.

**Drug Distribution in Mice**——JCL-ICR mice weighing about 30 g were used in all experiments. $^3$H-5-FU was injected into mice through tail vein, in 0.5 ml solution (0.9% NaCl solution), 0.5 ml suspension of albumin microspheres, or 0.25 ml suspension of albumin macroaggregates. Each mouse received 5 mg of microspheres or 0.625 mg of macroaggregates. The mice were sequentially killed by decapitation over a 3-day period. At the time of sacrifice, the liver, spleen, kidney, and lung were excised, and immediately weighed and burned on a sample oxidizer (Aloka, Model ASC-111). The tritium samples collected from the oxidizer were measured for radioactivity with a liquid scintillation counter (Aloka, Model LSC-651).

**Experimental Tumor System in Mice**——Ehrlich carcinoma ascites were transplanted intraperitoneally to ICR mice (both males and females, avg wt: 30 g) at every ten days. This treatment was the inoculation with Ehrlich ascites $2\times10^7$ cells/0.25 ml. Ehrlich solid carcinoma was prepared with the method which Ehrlich ascites ($2\times10^7$ cells/0.25 ml) were injected subcutaneously into the scapular region of male mice. The mice were used for the experiment tenth day after the injection of Ehrlich ascites.

## Results and Discussion

### (1) Physicochemical Property of Drug-entrapped Microsphere

The shape of albumin microspheres was invariably spherical probably due to the preparation method which the inner albumin phase of w/o emulsion was immediately solidified at high temperature. Figure 1 shows the scanning electron microphotograph of microspheres. The photograph was taken after the metal coating was carried out on microspheres with an ion-coater. (Eiko Seiki, Model 1B-3)

The size distribution of albumin microspheres was determined with a Model Zb Coulter Counter. Figure 2 shows the size distribution of albumin microspheres prepared at 180°. The most frequently occurring diameter varied between 0.4 and 1.0 $\mu$, and average diameter was 0.66 $\mu$. The size distribution of microspheres was mainly depending on the particle size of emulsion in the first step of the preparation of microspheres, and that particle size was influenced by the concentration of emulsifier and mechanical agitation.[12]

The microsphere is physically and chemically stable. After 5-FU powder was heated at 180° for 10 min, we determined the weight changes of the powder, UV spectrum of 5-FU in an acetate buffer (pH 4.7), and absorbance of the 5-FU solution at 266 nm. The evidence

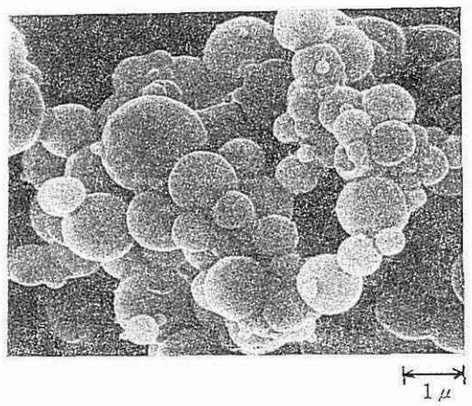


Fig. 1. Scanning Electron Microphotograph of Albumin Microspheres prepared at 180°

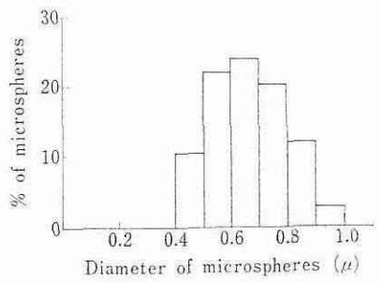


Fig. 2. Particle Size and Distribution of Albumin Microsphere prepared at 180°

12) M. Koishi, N. Fukuhara, and T. Kondo, *Chem. Pharm. Bull.* (Tokyo), **17**, 804 (1969).



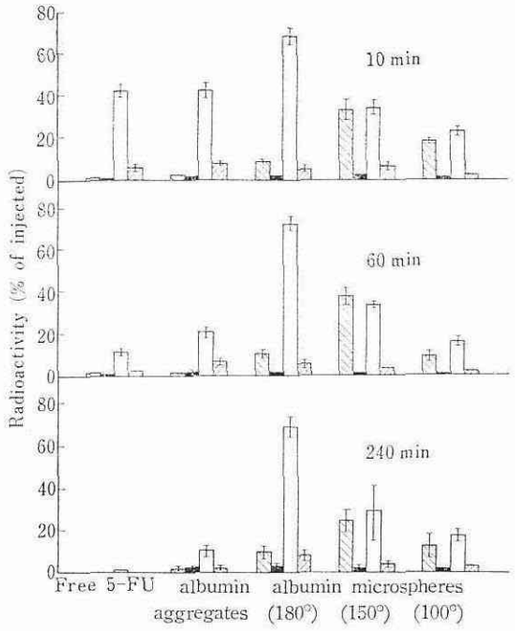


Fig. 3. Time Course of Tissue Distribution of Radioactivity after Intravenous Injection of Free 5-Fluorouracil, Albumin Macroaggregates, or Three Types of Albumin Microspheres

▨: lung, ■: spleen, □: liver, ▨: kidney
Each column represents the mean value of at least three measurements. Vertical bars indicate ±S.E.

of degradation of 5-FU by heating was not found in the results. When the albumin microspheres were prepared by the method described previously, about 4% of 5-FU present in original emulsion was associated with the microspheres. In contrast, entrapment of 5-FU in albumin macroaggregates was 6%.

### (2) Tissue Distribution in Mice

Figure 3 shows the distribution of radioactivity to various organs at 10, 60, and 240 min after intravenous injection of free $^3$H-5-FU, three types of albumin microspheres or albumin macroaggregates. In the figure, the distribution is represented as % of dose per whole tissue. Soon after injection of non-entrapped $^3$H-5-FU, most radioactivity was removed from the circulation and some of it transiently was in the liver and kidney. But radioactivity in the liver decreased from 42.7% (10 min) to 1.0% (240 min). In contrast, albumin macroaggregates entrapped drug was accumulated mainly in the liver (about 40% at 10 min), and 10% of dose were measured in the liver at 4 hr. The macroaggregates have been utilized as a scanning agent, and they are phagocytized by reticuloendothelial system of the liver. In case of albumin microspheres solidified at 180°, about 70% of dose were localized in the liver. This value was much higher than the radioactivity in the liver following injection of free drug or macroaggregate albumin including drug. The microspheres prepared at 180° might be delivered into the reticuloendothelial system because of its high phagocytic activity, as well as the distribution following injection of the macroaggregates. The temperature at which the albumin microspheres were prepared effects bio-distribution of radioactivity after intravenous injection. The microspheres prepared at 150° or 100° were accumulated not only in the liver but also in the lung, as shown in Fig. 3. Zolle, *et al.*[9a] pointed out that when the microsphere was suspended in a solution, swelling occurred, and the degree of swelling depended on the temperature at which the microspheres were prepared. This swelling of microsphere reflected in the accumulation in the lung, and the liver as well. And the change, that is increasing in size of the microspheres, occasionally occurs to obliteration of pulmonary vessels and pulmonary embolism.[13,14] Such localization of entrapped drugs in the liver suggested that albumin microspheres are potential drug carriers in chemotherapy.

Furthermore, we studied the drug carrier property of microsphere. The time courses of the disappearance of radioactivity in the liver and lung from 10 min to 3 day following

13) H.N. Wagner, Jr., D.C. Sabiston, Jr., J.G. McAfee, D. Tow, and H.S. Stern, *New Engl. J. Med.*, **271**, 377 (1964).
14) J. Szymendera, O. Mioduszewska, I. Licinska, A. Czarnomska, and B. Luska, *J. Nucl. Med.*, **18**, 478 (1977).

administration of three types of microspheres in mice are shown in Fig. 4 and 5. The disappearance of radioactivity in the liver was very slow. And the elimination of radioactivity from the lung was faster than that from the liver. Amount of drug accumulated and the elimination rate depended on the temperature at which the microspheres were prepared. The elimination of radioactivity after injection of microsphere prepared at 180° was slower than that after injection of spheres fixed at low temperature. Other work from our labolatory has shown that *in vitro* drug release from the microsphere entrapped 5-FU continued over a week, although the release rate was slow.[15] Therefore, these microspheres in the body may be disintegrated gradually and 5-FU entrapped in microspheres may be released. These results suggest that the albumin microspheres are instructive drug carriers with sustained drug release property, and it is expected that the microspheres are useful for maintenance of clinical effectiveness or therapeutic concentration in the tissue. Furthermore the release rate might be controlled by combination of three types of microspheres.

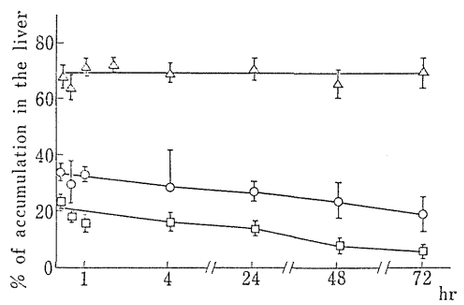


Fig. 4. Time Course of Drug Amount in the Liver after Injection of Microsphere prepared at 180° (△), 150° (○) 100° (□)

Each point represents the mean value of at least three measurements. Vertical bars indicate ±S.E.

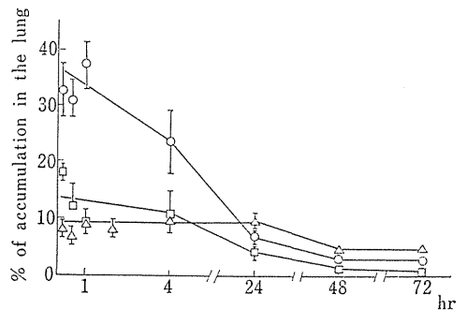


Fig. 5. Time Course of Drug Amount in the Lung after Injection of Microsphere prepared at 180° (△), 150° (○) or 100° (□)

Each point represents the mean value of at least three measurements. Vertical bars indicate ±S.E.

### (3) Effect of Pretreatment with Non-entrapped Microspheres on the Tissue Distribution of Drug-entrapped Microsphere

We studied the effect of pretreatment with non-entrapped albumin microsphere on the tissue distribution of drug-entrapped microsphere. Non-entrapped microspheres and drug-entrapped spheres were prepared at 180°. After the mice were pretreated with administration of non-entrapped microspheres (5 mg/0.5 ml), drug-entrapped microspheres (5 mg/0.5 ml) were injected at 5 min. Figure 6 shows the tissue distribution at 30 min following injection of drug-entrapped microspheres. Amount of drug accumulated in the lung of microsphere-pretreated mice increased and the uptake in the liver and spleen decreased, compared with a control (without pretreatment). Because the phagocytosis of the microspheres in the liver and spleen might be saturated after first injection of the microspheres (non-entrapped microspheres), the amount of drug accumulated decreased following second injection of the microspheres (5-FU entrapped spheres). In contrast, since the phagocytosis in the liver and the spleen was saturated, the uptake in the lung might gradually proceed. As the phagocytic activity in the lung may be low, it is doubtful that uptake in the lung occurs only by phagocytosis. In order to clarify the uptake mechanism, we are now investigating the histological study in some tissues after injection of the microspheres in mice.

15) K. Sugibayashi, M. Akimoto, Y. Morimoto, T. Nadai, and Y. Kato, *Life Sci.*, in preparation.



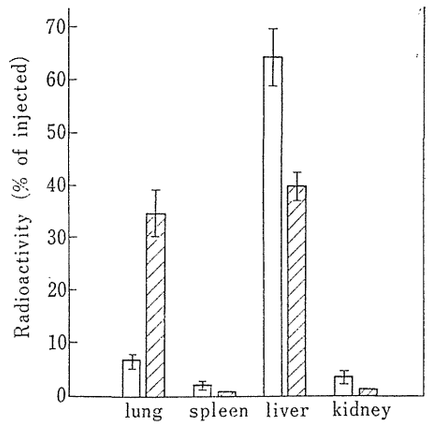


Fig. 6. Effect of Pretreatment with Non-entrapped Microsphere on the Tissue Distribution of Drug-entrapped Microsphere

□: control
▨: pretreated with non-entrapped microsphere
Each column represents the mean value of at least three measurements. Vertical bars indicate ±S.E.

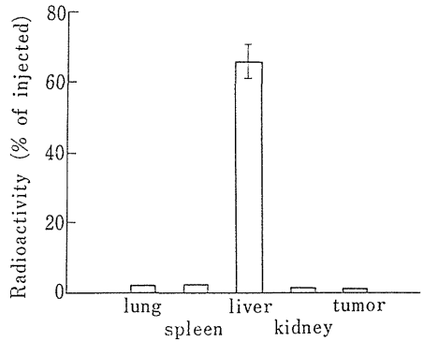


Fig. 7. Tissue Distribution of $^{3}$H-5-Fluorouracil entrapped in Microspheres at 30 min after Injection in Tumor Bearing Mice

Each column represents the mean values of four measurements. Vertical bars indicate ±S.E.

### (4) Application to Tumor Bearing Mice

In order to study the effect of the albumin microspheres on Ehrlich solid carcinoma in mice, we measured the radioactivity in the tumor tissues after injection of $^{3}$H-5-FU entrapped in albumin microsphere prepared at 180° (5 mg/0.5 ml) into tumor bearing mice. Figure 7 shows the tissue distribution of tumor bearing mice. The microspheres were not preferentially localized in the tumor cells, and most of the radioactivity was accumulated in the liver.

Gregoriadis, *et al.*[16] found that a degree of preferential uptake of liposome was observed in malignant deposits in most organ. Such preferential localization of liposome could be explained by an increased accessibility of the diseased tissue to liposomes due to extensive local vascularization or to an increased endocytic activity of tumor cells, or both. But Newton[17] suggested that access of dye penetration to tumor was poor. The uptake mechanism of drug or drug-entrapped carrier to tumor cells should not be a simple process, but a complex process which varied with the different tumor cells. We will study whether the albumin microspheres are valid to several experimental tumor systems, especially hepatoma cells. But if target tissue is in the liver, the albumin microsphere is useful drug carrier with its selectivity for the liver and prolonged action. We will make a study of possibility to direct drug-entrapped albumin microspheres to target tissues other than the liver through specific manipulations of the microsphere surface.

16) G. Gregoriadis, C.P. Swain, E.J. Wills, and A.S. Tavill, *Lancet*, **1**, 1313 (1974).
17) K.A. Newton, *Brit. J. Radiol.*, **38**, 224 (1965).

Communications to the Editor

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# Drug-carrier Property of Albumin Microspheres in Chemotherapy. I. Tissue Distribution of Microsphere-entrapped 5-Fluorouracil in Mice[1]

After intravenous injection in mice, 5-fluorouracil-6-$^{3}$H entrapped in albumin microspheres localized mainly in the liver, and the disappearance rate of radioactivity in microspheres from the tissue was very slow in comparison with that of free drug. Such preferential localization and sustained release of entrapped drugs suggested that albumin microspheres are useful as drug-carrier in chemotherapy.

**Keywords**——albumin microsphere; antitumor agent; drug-carrier; reticuloendothelial system; drug distribution

It is desirable that the drug selectively reaches its target tissues in drug therapy. After the drug is absorbed or injected into the blood stream, it may be distributed into the interstitial, cellular, and transcellular fluids. The rate, extent, and pattern of the distribution are determined by the physicochemical characteristics of the drug. And the undesired effects of the drug sometimes arise because of the accumulation in other tissues except for target tissues. In particular, since most antitumor agents lack tumor specificity thereby resulting in dose-limiting systemic toxicity, these drugs should be delivered to target tissues correctly.

Past approaches to find method of directing drug-carrier to paticular tissues have been largely related to drug-containing liposome (phospholipid vesicles). Gregoriadis and his coworkers[2] reported that liposome-entrapped drugs intravenously injected into rats were concentrated at the liver and the spleen by the phagocytosis of the reticuloendothelial system. Tanaka, *et al.*[3] also reported similar results that liposome-entrapped $^{14}$C-inulin after intravenous injection were recovered mainly in the liver and the spleen. Trouet, *et al.*[4] reported reduced toxicity and increased effectiveness in the treatment of leukemia when DNA complexes of the antitumor agent daunomycin are pinocytized from solution. Radiologists utilized the phagocytic activity of the liver and the spleen to study and diagnose the function of the reticuloendothelial system by using radiolabeled albumin aggregates, albumin microspheres and sulfur colloids.[5] Kramer[6] suggested the possibility that albumin microspheres could be utilized as the prominent drug-carrier with tissue specificity. Drug-carrier property of albumin microspheres in chemotherapy also appeared to be of interest to us. In this paper we wish to report the tissue distribution of 5-fluorouracil (5-FU) entrapped in albumin microspheres after intravenous injection in mice. Albumin microspheres are physically and chemically stable, are selectively removed from blood stream by the reticuloendothelial system, and are nonantigenic and metabolizable within the body. Wagner, *et al.*[7] reported

1) Part of this work was presented at 97th Annual Meeting of Pharmaceutical Society of Japan, Tokyo, April, 1977.
2) *a*) G. Gregoriadis and B.E. Ryman, *Europ. J. Biochem.*, 24, 485 (1972); *b*) G. Gregoriadis, *FEBS Letters*, 36, 292 (1973).
3) T. Tanaka, K. Kobayashi, K. Okumura, S. Muranishi, and H. Sezaki, *Chem. Pharm. Bull.* (Tokyo), 23, 3069 (1975).
4) A. Trouet, D.D. Campeneere, and C. De Duve, *Nature* (London), 239, 110 (1972).
5) *a*) I. Zolle, B.A. Rhodes, and H.N. Wagner, Jr., *Int. J. Appl. Radiat. Isotop.*, 21, 155 (1970); *b*) U. Scheffel, B.A. Rhodes, T.K. Natarajan, and H.N. Wagner, Jr., *J. Nucl. Med.*, 13, 498 (1972).
6) P.A. Kramer, *J. Pharm. Sci.*, 63, 1646 (1974).
7) H.N. Wagner, Jr., D.C. Sabiston, Jr., J.G. McAffee, D. Tow, and S. Stern, *New Engl. J. Med.*, 271, 377 (1964).

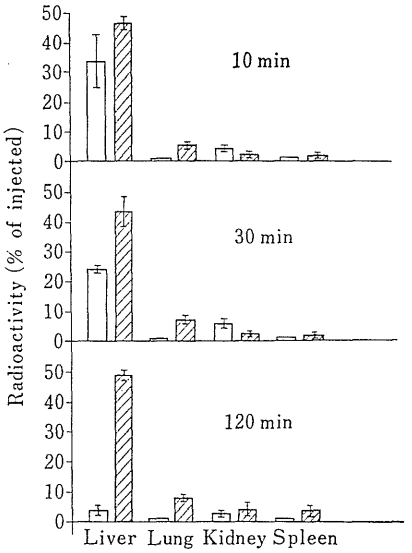


Fig. 1. Time Course of Uptake and Persistence of 5-Fluorouracil-6-$^3$H in Tissues

▨: microsphere-entrapped 5-fluorouracil.
□: non-entrapped 5-fluorouracil.
Each column represents the mean value of 3—5 experiments.
Vertical bars indicate S.E.M.

that they administered aggregated albumin in more than 1200 studies in over 300 subjects and were unable to detect any evidence of antigenicity. Therefore the results satisfy the criteria which a substance should possess if it is to be satisfactorily employed as drug-carriers.

Bovine serum albumin microspheres containing the antitumor agent 5-fluorouracil were prepared by a modification of the method of Scheffel, *et al.*[5b] Size distribution of microspheres was determined with a coulter counter and the main part was in the range 0.4—1.0 microns.

In animal experiment, 5-fluorouracil-6-$^3$H was injected into the mouse through the tail vein (ICR, 22—32 g), as 0.5 ml of a solution (0.2% polysorbate 80 in 0.9% NaCl solution) or a suspension of microspheres. And mice were killed at certain time intervals. Radioactivity in the lung, liver, spleen, and the kidney was determined by using a sample oxidizer and a liquid scintillation counter.

Soon after injection of non-entrapped 5-fluorouracil-6-$^3$H, most radioactivity was removed from the circulation and some of it transientry was in the liver and the kidney. But radioactivity in the liver decreased from 34.30% (10 min) to 3.14% (120 min). In contrast, entrapped 5-fluorouracil-6-$^3$H was mainly accumulated in the liver, and a considerable amount of the drug accumulated was still retained by the tissue 120 min later as shown in Fig. 1. This is similar to a phenomenon that albumin macroaggregates as scanning agent in clinical use today are phagocytized by the reticuloendothelial system of the liver. The albumin microspheres also are delivered into the reticuloendothelial system because of its high phagocytic activity. These results suggested that entrapment of the drug in albumin microspheres led to a dramatic change in its retaining in the liver.

After the microspheres were administered into the mice, disappearance of the radioactivity in the liver was very slow. Other work from our own laboratory has shown that *in vitro* drug release from microspheres entrapped 5-fluorouracil continued over a week, although the release rate was slow. From these results, the microspheres in the body may be disintegrated gradually and 5-fluorouracil entrapped in microspheres may be released. And it is expected that the albumin microspheres are useful for maintenance of the clinical effectiveness or therapeutic concentration in the tissue. Such good localization and prolonged action of entrapped drugs in the liver suggested that albumin microspheres are potential as drug carrier in chemotherapy. Work is now in progress on the effect of albumin microspheres on the tumor bearing mice.

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# Spectrophotometric Analysis for Fluorouracil in Biological Fluids

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Fluorouracil (5-Fluorouracil; 5-FU) is a monoanion at pH = 10 and forms an ion-pair complex with a quaternary alkylammonium ion. *De Leenheer* and co-workers [2] reported that the ion-pair complexes might be quantitatively extracted from aqueous solutions into organic solvents, and they spectrophotometrically determined the concentration of 5-FU. But as their method used column chromatography, it was cumbersome and time-consuming.

Here we present a more rapid chemical assay of 5-FU by modifying the principle of their method. The applicability of this method was shown by determining the plasma and tissue levels of 5-FU in mice.

**Spektrofotometrische Bestimmung von Fluorouracil aus biologischen Flüssigkeiten**

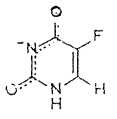
Fluorouracil (5-Fluoruoracil; 5-FU) ist bei pH = 10 ein Monoanion und bildet Ionenpaar-Komplexe mit quarternisierten Alkylammoniumionen. *De Leenheer* und Mitarb. [2] berichteten, daß Ionenpaar-Komplexe quantitativ aus wäßriger Lösung in ein organisches Lösungsmittel extrahiert werden können. 5-FU ließ sich so spektrofotometrisch bestimmen. Doch ist die Methode durch Verwendung der SC zeitaufwendig.

Die hier beschriebene Methode zeichnet sich durch Einfachheit und Genauigkeit aus. Das fotometrische Verfahren kann zur Bestimmung des Plasma- und Organspiegelverlaufes benutzt werden. Es wird unter dem Aspekt des Einsatzes für einen Therapieservice geprüft.

## 1. Introduction

Monitoring plasma and tissue concentration is required not only for antibiotics but also for antitumor agents so as to rationalize a method of administration, to strengthen the therapeutic effects of the drug, and to diminsh the side effects. In addition, so as to develop a new drug-delivery system of antitumor agents, the measurement of plasma and tumor tissues drug concentration may be required. We have studied the drug carrier property of albumin microspheres containing fluorouracil (5-FU) [7-9]. In this experiment we wish to determine the unchanged 5-FU level in aqueous biological materials. The assay using radioactively-labeled drug [7, 8] and bioassay using antimicrobial of 5-FU [4] must interfere very little with the endogenous substances and have good sensitivity. But the estimation of drug amounts with these methods shows variability due to metabolism and lacks specifity, while the spectrophotometric assay [1] and the chromatographic analysis of TLC [3] and GLC [5] etc. are convenient methods but lack the sensitivity and rapidity needed for assay of 5-FU. Therefore these determination methods are not suitable to monitor the blood and other body fluid concentrations of this drug so as to rationalize the dose schedule for cancer patients. Biopharmaceutical studies of this drug have been hampered by the lack of a suitable chemical assay.

Recently, *De Leenheer* and co-workers [2] reported a spectrophotometric determination which is comparably sensitive to free 5-FU. As 5-FU is insoluble in chloroform, ethylether and other organic solvents, the isolation of 5-FU from aqueous biological materials into an organic solvent is difficult. But they used the phenomena that 5-FU was a monoanion at pH = 10 and might be extracted from aqueous solution with quaternary alkyl ammonium ions into an organic solvent as the ion-pair, and then overcame the difficulty that 5-FU is only slightly extracted into an organic solvent.



1

$[CH_3(CH_2)_4]_4N^+$

2

In their method, however, when the ion-pair complex was extracted into an organic solvent, they used a glass column-packed cellulose. And this adsorbent must be purified in a column by washing with ethanol and dichloromethane until the absorbance of the eluate at 269 nm was constant at 0.010. These procedure and column extraction are somewhat cumbersome and time-consuming. We therefore developed a more rapid and equally sensitive method a determination of 5-FU in some biological samples by modifying their method.

## 2. Results and Discussion

5-FU is slightly soluble in water (solubility = 12.20 mg/ml at 25 °C [6]) and a diprotic acid ($pKa_1 = 8.0 \pm 0.1$, $pKa_2 = 13.0 \pm 0.1$ [6]). As it is insoluble in many organic solvents, it is impossible to extract free 5-FU from an aqueous biological system into some organic solvents. But monoanion species of 5-FU [1] form a 1:1 ion-pair complex with tetrapentylammonium monocation [2], and the complex may be extracted into organic solvents. Because the spectral properties of the ion-pair in the organic phase were not accurate, a back extraction was performed with a buffer solution which included a much stronger ion-pair former, $ClO_4^-$. At this time all 5-FU originally present as ion-pair in the organic phase froms monoanionic ion and may be quantitatively transfered into the aqueous phase. At the same time, the quaternary ammonium cation forms an ion-pair complex with a perchlorate anion and is stable in the organic solvent. 5-FU anion which is back extracted into the buffer layer gives good spectral properties at 269 nm.

In this assay it is important to adjust pH in the biological sample to 10. If the buffer capacity is weak and the pH of sample added to the buffer solution is not 10, then the ionic strength of the buffer solution must be increased.

In this determination, 5-FU may often be obscured by endogenous substances. If high blank values were obtained, then deproteinization should be carried out. The detailed procedure is as follow: One ml of biological sample (sample containing 0-100 $\mu$g 5-FU) was put into a centrifuge tube, 1 ml of NaOH (2 mol/l) and 1 ml of 10% zinc sulfate solution were added and the protein was denaturated. Then after this sample was neutralized with 1 ml of 10% phosphoric acid and centrifuged, 3 ml of the supernatant was used for the determination of ion-pair method. If hydrochloric acid was used as the neutralization reagent, 5-FU was not extracted into an organic phase. If trichloroacetic (TCA) was used as the deproteinization reagent, 5-FU was not extracted, because the chloride anion and TCA anion form an ion-pair complex stronger than the 5-FU anion with a quaternary ammonium cation. It appears from these facts that it is not suitable to use hydrochloric acid as the neutralization reagent and TCA as the deproteinization reagent.

Hemolysis and remaining blood affect the absorbance of a blank and the sensitivity of this analysis. In collecting blood samples, hemolysis should be avoided and in extraction of some organ tissues, the blood remaining in tissues should be cleared. After the mice were sacrified by decapitation and as much blood as possible in the body was drawn out, the tissues should be extracted. Their extracted samples should be washed well with 0.9% NaCl solution. A deproteinization procedure may be effective against the hemolysis and remaining blood, but the sensitivity may be reduced by increasing the reagent vo-

lume. If a biological sample contains much remaining blood, 5-FU in the sample can not be determined due to high absorbance of the blank.

Linear standard curves (Fig. 1) were prepared corresponding to 5-FU concentrations from zero to 100 μg/ml in aqueous solution; then plasma and liver samples were obtained and used to determine the drug levels of samples in this concentration range after accounting for any dilution changes. The plasma blank is almost zero ~~the~~ same as the aqueous solution. A blank value of the tissue sample is not negligible, but this value is constant in many samples. As to the sensitivities of plasma and tissue samples, they are similar to as aqueous solution as shown by the slope of the straight line in Fig. 1.

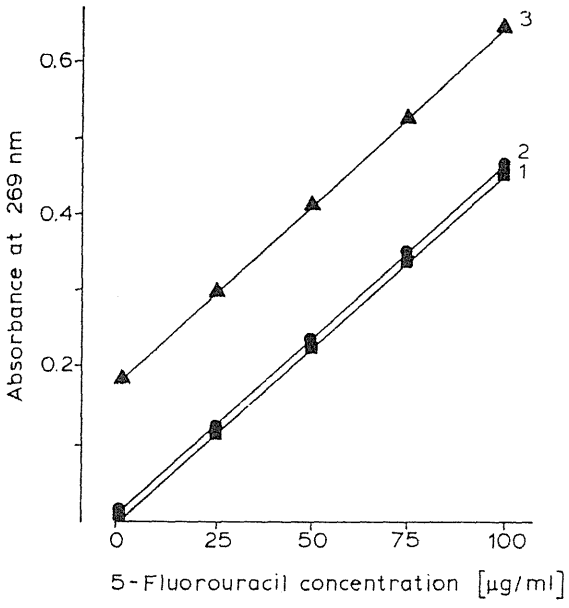



Fig. 1. Calibration curves for 5-fluorouracil in aqueous solution (1) plasma (2) and supernatant of liver homogenate (3)

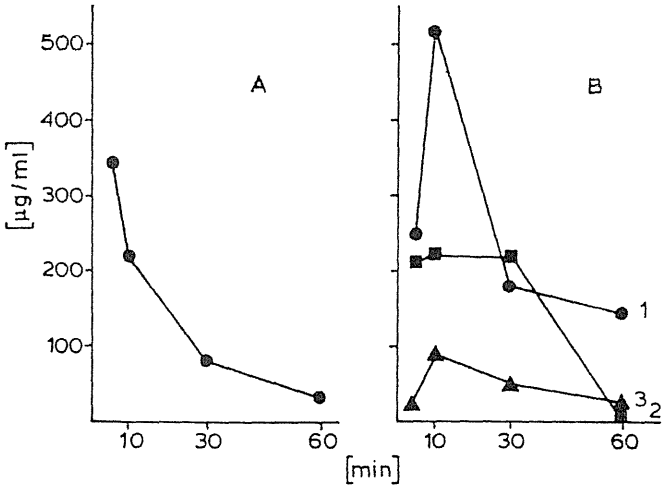



Fig. 2. Plasma and tissues concentration of 5-fluorouracil in mice given a single injection of 1.25 g/kg. A: plasma, B: tissues-kidney (1), lung (2), liver (3). Each point was determined after a mouse was sacrified at 5, 10, 30, and 60 min. Blanks of plasma ~~or~~ and tissues were ~~ob~~ obtained from an un-treated mouse

Next the applicability of this method was carried out by determining plasma and tissues levels of 5-FU. Fig. 2 illustrates the examples of the plasma and tissues time course of the drug in mice receiving 1.25 g/kg by i. v. push.

This analytical procedure is a specific method for 5-FU, and is little obstructed by its metabolites. And this is so rapid and good sensitive that it is possible to estimate 5-FU concentration in many manufactured dosage forms and other biological samples. (urine, bile, saliva and feces etc.) And this method is convenient for a routine assay of 5-FU as clinical studies in cancer patients.

## 3. Experimental

### *3.1. Chemicals and Reagents*

5-FU was supplied from Kyowa Hakko Co., Ltd. and tetrapentylammonium iodide was obtained from Eastman Kodak Co., Ltd. Tetrapentylammonium counterion was prepared by the method described by *De Leenheer* and coworkers [2] with a slight modification. All of the other chemicals were of reagent grade and without further purification.

### *3.2. Ion-pair Extraction Method*

Each 1 ml of biological material (see 3.3.) was put into a 10 ml glass-stoppered centrifuge tube, and 0.2 ml of concentrated carbonate buffer (0.625 mol/l), pH = 10 and ionic strength 2.5, and 1 ml of tetrapentylammonium counterion were successively added and then shaken. Then 5 ml of $CH_2Cl_2$ was added and shaken for 10 min. After centrifugation, 4 ml of the organic layer was pipetted off and shaken for 10 min with 5 ml of $NaClO_4$ (0.2 mol/l) in carbonate buffer (0.025 mol/l), pH = 10 and ionic strength 0.1. The concentration of the buffer layer was determined spectrophotometrically at 269 nm by a Hitachi Type 100-20 spectrophotometer.

### *3.3. Plasma and Tissue Sample Preparation*

5-FU 1.25 g/kg solution prepared by dissolving the drug in trisaminomethane (0.7 mol/l) was injected into the ICR mouse through the tail vein, and the mice were sacrified by decapitation at certain time intervals; 0.5 ml blood and some tissues (liver, kidney and lung) were removed. The blood sample was quickly transfered into a 10 ml centrifuge tube previously containing 1 ml of 3.8% sodium citrate solution, and plasma was collected in the usual way. After each tissue was washed quickly in 0.9% NaCl solution, these samples were homogenated with nine or nineteen times as 0.9% NaCl solution as a tissue weight by Potter-Elvehjem homogenizer. The 10 or 5% homogenate was centrifuged at 4000 rpm for 10 min; 1 ml of supernatant was used for ion-pair extraction.

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# DRUG-CARRIER PROPERTY OF ALBUMIN MICROSPHERES IN CHEMOTHERAPY. III. EFFECT OF MICROSPHERE-ENTRAPPED 5-FLUOROURACIL ON EHRLICH ASCITES CARCINOMA IN MICE

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To examine the possibility of utilizing albumin microspheres as drug-carriers, an *in vitro* release of 5-fluorouracil (5-FU) from albumin microspheres was examined and the effect of intraperitoneally injected drug-carrying microspheres on Ehrlich ascites carcinoma in mice was studied. *In vitro* release characteristics determined by dialysis experiments showed that 5-FU release continued over one week. We also noted that drug release in the peritoneum of ascites-bearing mice continued over one week and that their life-span increased. Furthermore, the microspheres were phagocytized *in vivo* by the ascites cells. Our results suggest that albumin microspheres containing 5-FU may represent an effective system of drug delivery with prolonged action.

**Keywords**——albumin microsphere; drug-carrier; 5-fluorouracil; prolonged action; phagocytosis; tumor cell

Inert carriers have been used in chemotherapy to direct drugs to target tissues[1] and they have also been utilized to sustain the release of pharmaceuticals.[2] Miller *et al.*[3] showed that polylactate and polyglyconate copolymers were degraded gradually after intraperitoneal injection into rats, and they suggested that the polymers were useful as drug carriers. Arakawa *et al.*[4] reported sustained drug release from liposome suspensions (phospholipid vesicles) and Gregoriadis *et al.*[5,6] suggested that liposome-entrapped drugs were phagocytized preferentially by tumor tissues. These experiments showed the liposome to be an effective drug carrier in cancer chemotherapy. Albumin microspheres have been utilized as a lung or liver scanning agent.[7,8] Kramer suggested the possibility that albumin microspheres could be utilized as effective, tissue-specific drug carriers,[9] and he and his coworkers reported that human serum albumin microspheres containing 6-mercaptopurine were selectively phagocytized *in vitro* by HeLa and glioblastoma cells.[10]

We recently reported that 5-fluorouracil (5-FU) entrapped in bovine serum albumin (BSA) microspheres was present in high levels in the liver of mice after intravenous injection.[11] But it is obscure that albumin microspheres may represent therapeutic effect with prolonged action in cancer chemotherapy. If albumin microspheres containing antitumor agent show the sustained release and prolonged action, then albumin microspheres may provide a new field in cancer therapy. Then we study the sustained release of BSA microsphere-entrapped 5-FU and the prolonged action of the entrapped drug in Ehrlich ascites carcinoma as a model tumor *in vivo*. This preliminary study was undertaken to determine the extended action of the microspheres, to measure the sustained release of 5-FU from microspheres, and to observe the phagocytosis of microspheres by tumor cell *in vivo*.

## MATERIALS AND METHODS

*Materials*——5-Fluorouracil (5-FU) was obtained from Kyowa Hakko Co., Ltd. and bovine serum albumin (BSA) from Seikagaku Kogyo Co., Ltd. ICR mice were used in all animal experiments. Male and female ICR mice, weighing approximately 30 g were intraperitoneally inoculated at 10-day intervals with $2\times10^7$ Ehrlich ascites.

*Preparation of BSA Microspheres*——BSA microspheres containing the antitumor agent 5-FU were prepared by a modification of the method of Scheffel *et al.*[8] The final microspheres contain about 3.3% 5-FU.

*In Vitro Drug Release*——Drug release from the microspheres was determined by a dynamic dialysis system with a cellulose tubing (36/32, Visking Co.) (Fig. 1). One hundred milligrams BSA microspheres containing 5-FU were suspended in isotonic phosphate buffer (pH 7.4). After 10 min ultrasonication using a 2.6 cm titanium probe at 250 $\mu$A in a sonicator (Nihonseiki Seisakusho, Model G50022-4) and centrifugation to remove the drug which adhered to the microspheres, the precipitates were resuspended with 3 ml of isotonic phosphate buffer (pH 7.4) and dialyzed against of 47 ml of isotonic phosphate buffer at 37°. The inner solution was stirred at 50 rpm with a stirrer attached to an electric motor and the outer solution was stirred at 200—300 rpm with an acrobat stirrer (Emuesukiki Co., Ltd.).

Samples (1 ml) were withdrawn from the outer solution at certain intervals and 1 ml of buffer was added to keep the volume constant. Drug adsorption to the Visking dialysis tube was negligible. The drug concentration of the samples was determined at 266 nm by a spectrophotometer (Model 100-20, Hitachi Co., Ltd.) after the addition of acetate buffer (pH 4.7).[12]

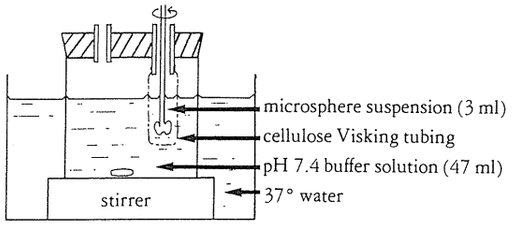



FIG. 1. *Apparatus used to Study the Release of 5-FU from Microspheres*

*Toxicity Studies*——Toxicity of the microspheres containing 5-FU was determined by animal survival and evidence of rejection in 30 g male mice following intraperitoneal injection. Acute (7 days) and chronic (30 days) evaluations of the toxic effects were performed in 20 mice per group.

*Microscopic Study of Phagocytosis*——Phagocytic uptake was determined as follows: 24 hr after $2\times10^7$ ascites inoculation, the mice received an intraperitoneal injection of microsphere (30 mg of microsphere containing 1 mg of 5-FU per mouse) or 0.9% NaCl solution (control) and were killed by cervical dislocation 5 days after inoculation. The ascites cells were collected immediately, fixed in methyl alcohol, stained with Giemsa, and examined under an optical microscope. Alternatively, dehydration, drying and metal-coating of the ascites were carried out, and then the samples were used to take scanning electron micrographs.

*In Vivo Drug Release*——To determine drug release in the peritoneum and phagocytic uptake by Ehrlich ascites carcinoma, 24 hr after inoculation with Ehrlich ascites cells, the mice were administered with intraperitoneal injection of

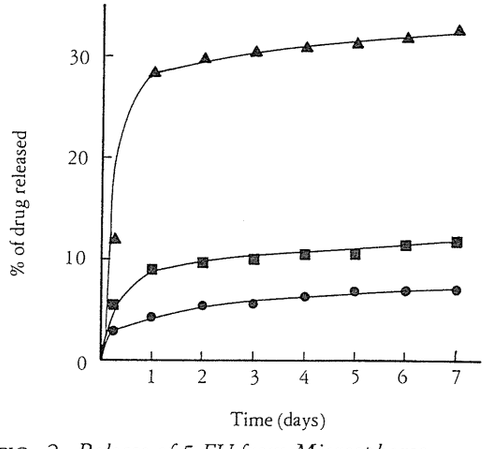


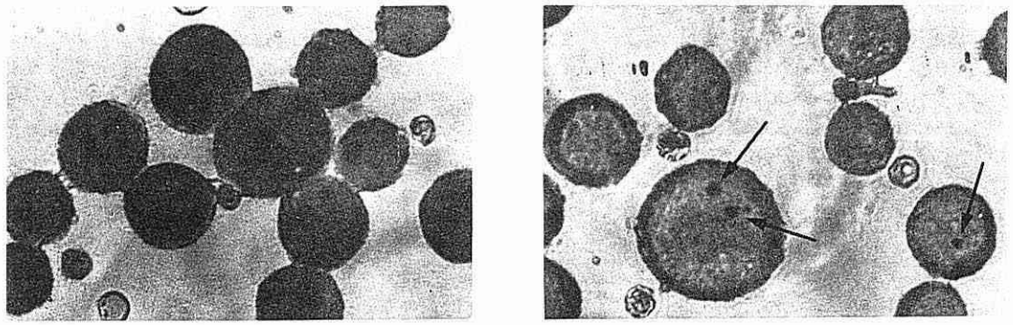

FIG. 2. *Release of 5-FU from Microspheres Albumin microspheres prepared at 180° (●), 150° (■), 100° (▲).*

microspheres containing 5-FU (30 mg of microspheres containing 1 mg of 5-FU per mouse), free 5-FU (1 mg per mouse) or 0.9% NaCl solution (control). Changes in body weight and survival times of treated, tumor-bearing mice were recorded.

RESULTS

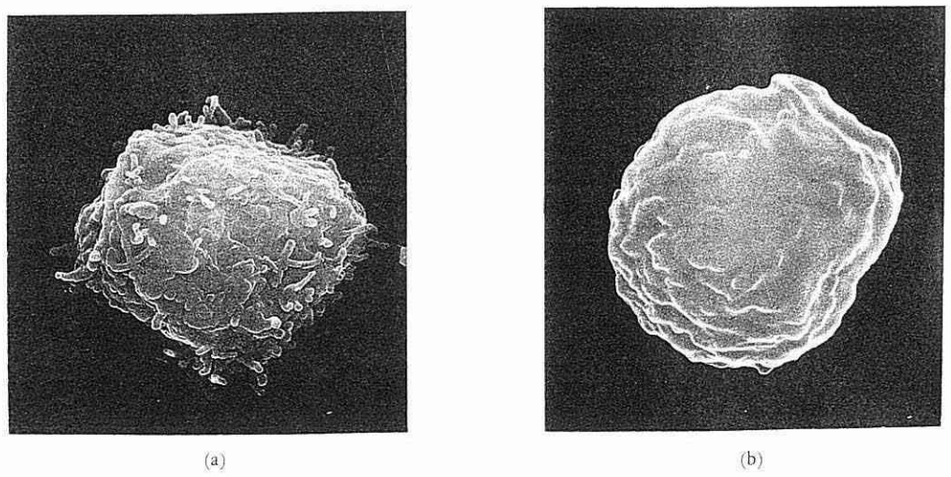
*In Vitro Drug Release*

As shown in Figure 2, *in vitro* 5-FU release continued over one week, although the release rate was very slow. The level of drug release depended on the temperature of the microsphere preparation, suggesting difference in microsphere structures and hardness levels. Zolle *et al.*[7] reported that swelling occurred in microspheres suspended in a solution, and that the degree of swelling decreased with increase in the temperature of the microsphere preparation. An increase in the temperature of the microsphere preparation increased the hardness of



(a) (b)

FIG. 3. *Micrographs of Ehrlich Ascites Carcinoma*
*(a) control, (b) after treatment with albumin microspheres. Arrows indicate microspheres phagocytized by tumor cells. Giemsa stain (magnification×600)*



(a) (b)

FIG. 4. *Scanning Electron Micrographs of Ehrlich Ascites Carcinoma*
*(a) control (×6200), (b) after treatment with albumin microspheres containing 5-FU (×14000).*



the microspheres, leading to decreased drug release. Consequently, drug diffusion in the microsphere matrix is reduced due to decreased microsphere porosity and increased tortuosity.

*Microsphere Toxicity*

Negligible side effects were discovered in acute and chronic toxicity studies. None of the 20 mice examined in each type of study died even the highest dosage tested (50 mg microsphere per mouse). No localized ulcerations and/or loss of hair, indicative of rejection, were observed at various dosage levels tested.

*Phagocytosis of Microspheres by Ehrlich Ascites Carcinoma*

Figure 3 shows micrographs of Ehrlich ascites carcinoma in mice 5 days after injection of albumin microspheres or 0.9% NaCl solution. Figure 3b reveals microsphere phagocytosis by the ascites cells. Kramer and Burnstein,[10] using isotope-labeled drugs entrapped in microspheres, demonstrated the uptake of albumin microspheres by tumor cells *in vitro*. Since the mechanism underlying phagocytosis of macromolecules and microspheres has not been completely elucidated to date, we attempted the additional demonstration of the presence of microspheres in the cell lysate.

Figure 4 shows that intraperitoneal administration of albumin microspheres containing 5-FU effected morphological changes on the surface of Ehrlich ascites cells compared with injection of

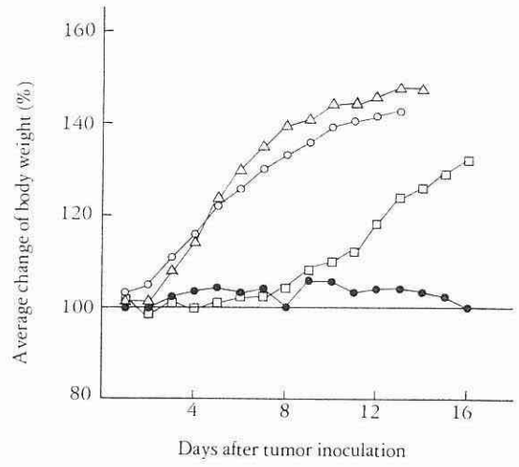



FIG. 5. *Change in Body Weight of Ehrlich Ascites Tumor Bearing Mice*
*Treatment with 0.9% NaCl solution (○), free 5-FU (△) and microspheres containing 5-FU (□) normal control bearing no tumor (●)*

0.9% NaCl solution. This change may be explained by a sustained abdominal level of 5-FU, by an intracellular concentration of 5-FU due to the endocytic activity of the tumor cells or by both effects.

*Effect of Microspheres Containing 5-FU on Ehrlich Ascites*

Although 5-FU has been found to be active against Ehrlich ascites carcinoma,[13] its effect was

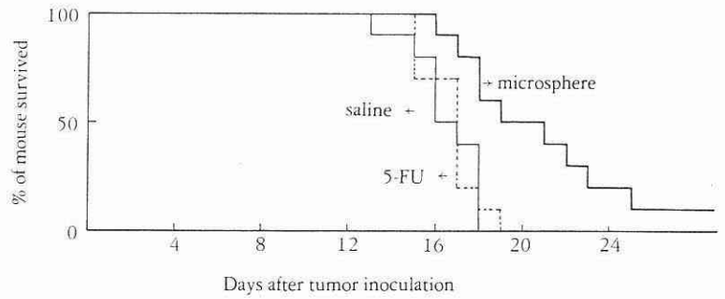



FIG. 6. *Effect of Albumin Microsphere-entrapped 5-FU on the Survival of Mice inoculated with Ehrlich Ascites Carcinoma*
*The life span was increased by 1.2% in 5-FU treated mice and 20.5%* in mice administered microspheres containing 5-FU.*
**This value represents the average of 9 mice.*

poor due to a fast elimination rate. We further examined the effect of microspheres containing 5-FU in Ehrlich ascites and found that intraperitoneal injection of albumin microspheres containing 5-FU resulted in drug activity against the ascites and the suppression of tumor growth at the inoculation site, lasting for approximately one week. Thereafter, however, tumor growth effected an increase in animal body weight (Figure 5). The increase in life-span effected by microsphere treatment is shown in Figure 6.

## DISCUSSION

Zolle *et al.*[7] and Scheffel *et al.*[8] utilized the phagocytic activity of the liver and spleen and radiolabeled albumin microspheres to study and diagnose the function of the reticuloendothelial system. Albumin microspheres were degradated and metabolized and no evidence of antigenicity was found, indicating the effective drug-carrier properties of microspheres. We reported earlier that microspheres injected intravenously into mice were concentrated in the liver due to phagocytosis of the reticuloendothelial system.[11 14]

In the present study we found that *in vitro* release of 5-FU from albumin microspheres continued over one week (Figure 2). Our findings that after microsphere injection, the average body weight of the ascites-bearing mice did not increase for one week, indicate that sustained drug release occurs in the peritoneum and that effective drug concentrations may be maintained for a week (Figure 5). We also noted a prolongation in the life-span of tumor-bearing mice following therapeutic microsphere administration (Figure 6). Our experimental results suggest that albumin microspheres containing 5-FU may represent an effective system of drug delivery with prolonged action.

However, suppression of tumor growth disappeared by one week. This may be due to the fact that the amount of 5-FU delivered in single-shot administration of microspheres was therapeutically insufficient.

Studies are presently under way in our labolatory to examine the effects of multiple dose administration of microspheres containing 5-FU. In addition, we plan to investigate the applicability of the microsphere drug delivery system to several experimental tumor systems.

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*J.Pharm. Dyn.*, 2, 356—364(1979)

# THE STUDY ON THE BIOLOGICAL FATE OF PARABEN AT THE DOSE OF PRACTICAL USAGE IN RAT. I. THE METABOLISM AND EXCRETION OF ETHYL *p*-HYDROXYBENZOATE (ETHYL PARABEN) AND *p*-HYDROXYBENZOIC ACID*

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The biological fates of ethylparaben (EP) which has been widely used as a preservatives for the pharmaceutical preparations and foods, and *p*-hydroxybenzoic acid (HB) which is the parent compound of EP, were investigated at the dose of practical usage (2 mg/kg) in rats. Although both EP and HB were metabolized to glycine conjugate (M1), ester type glucuronide (M3) and sulfate (M4) of HB and excreted in the urine and bile, the excretion ratios were different as compared with the results of higher dose experiments carried out by other authors, and a route dependency was also found in the rate of excretion in the bile.

The excretion data obtained in this study are shown as follows: In intravenous administration of EP, the excreted total activity was 91.3% per dose in the urine and 5.97% in the bile, and percentages per dose of the major metabolites excreted in the urine were 8.14% of HB, 39.6% of M1, 29.5% of M3 and 6.48% of M4. In intraduodenal administration of EP, the excreted total activity (83,5%) and the activity excreted as HB (3.51%) decreased compared with the intravenous administration.

In the HB administration, the route dependency of the total activity was not found, but a decrease in the excretion of HB in the intraduodenal administration was found.

The excretion of total radioactivity almost ceased by 5 hr and the biological half-lives obtained from the $\beta$-phase of the sigma-minus plots of the metabolites excreted in the urine were 40—70 min.

The result obtained in this study differ from those of other authors at the high dose experiments.

**Keywords**——biological fate; metabolism; excretion; paraben; ethyl *p*-hydroxybenzoate; *p*-hydroxybenzoic acid

Alkyl *p*-hydroxybenzoate (paraben) has been widely used as a preservative for pharmaceutical preparations, foods, and cosmetics.

Matthews *et al.* showed that parabens were safe preservatives based on the study in the acute and clinical toxicity.[1] On the biological fates, Tsukamoto *et al.* demonstrated that *p*-hydroxybenzoic acid (free acid, HB), *p*-hydroxyhippuric acid (glycine conjugate of HB, M1), *p*-carboxyphenyl glucuronide (ether type glucuronide of HB, M2), *p*-hydroxybenzoyl glucuronide (ester type glucuronide of HB, M3) and *p*-carboxyphenyl sulfate (ether sulfate of HB, M4) were excreted in the urine of the rabbit after oral administration of

* This work was presented at the 98th Annual Meeting of Pharmaceutical Society of Japan, Okayama, April 1978.

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28(10)3087—3092(1980)

# Drug-carrier Property of Albumin Microspheres in Chemotherapy. IV.[1] Antitumor Effect of Single-shot or Multiple-shot Administration of Microsphere-entrapped 5-Fluorouracil on Ehrlich Ascites or Solid Tumor in Mice[2]

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(Received April 18, 1980)

To examine the possibility of utilizing albumin microspheres containing 5-fluorouracil (5-FU) as a drug carrier, the antitumor activity of albumin microsphere-entrapped 5-FU against Ehrlich ascites carcinoma and solid tumor in mice was studied. After intraperitoneal injection of microsphere-entrapped 5-FU into Ehrlich ascites-bearing mice, the 5-FU level in the ascites was very high compared with that after injection of the free drug. The suppressive effect of microsphere-entrapped 5-FU on tumor growth in ascites-bearing mice was significantly higher than that of the free drug. After multiple-shot administration of microsphere-entrapped 5-FU to ascites-bearing mice, the increase in life-span was over 50% compared with a control. The therapeutic effect of microsphere-entrapped 5-FU on Ehrlich solid tumor after intratumoral injection was also studied. 5-FU level in the tumors of mice was significantly higher after intratumoral injection of microsphere-entrapped 5-FU than when the free drug was administered. The injection of microsphere-entrapped 5 FU, which was slowly released from the albumin microspheres in the solid tumor, caused a higher suppression of tumor growth at the inoculation site than administration of the free drug at the same dose. These results suggest that albumin microspheres containing 5-FU may represent an effective system for drug delivery, with prolonged action.

**Keywords**——albumin microsphere; drug carrier; 5-fluorouracil; Ehrlich ascites carcinoma; Ehrlich solid tumor; prolonged action; mouse

The effective use of pharmacologically active agents is often limited by side effects on the healthy tissue, incomplete transport to the desired site of action, and/or insufficiently sustained action after reaching the target tissue. This is particularly true in the chemotherapy of cancer. One of the methods used to overcome these shortcomings is to employ non-toxic and biodegradable drug carriers which direct drugs to the target tissues. From this point of view, various investigators have attempted to develop useful drug carriers, based on evaluation by *in vitro* and *in vivo* experiments.[4] The use of liposomes has been extensively investigated by Gregoriadis *et al.*[5] and their potential as carriers for pharmaceuticals *in vivo* seems clear. Kramer suggested that albumin microspheres, which have been used as a lung or liver scanning agent,[6] might be utilized as vehicles for achieving specific drug delivery,[7] and he

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2) Part of this work was presented at the 99th Annual Meeting of the Pharmaceutical Society of Japan, Sapporo, Aug., 1979.
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and his co-workers reported that human albumin microspheres containing 6-mercaptopurine were phagocytized by several tumor cell lines.[8]

We also have been studying the utility of albumin microspheres as drug carriers which might localize antitumor agents in target tissues. In previous studies, an enhanced accumulation of 5-fluorouracil (5-FU) entrapped in bovine serum albumin (BSA) microspheres was shown in mice.[9] Further, it was found that the injection of 5-FU after its entrappment in microspheres prolonged the survival of Ehrlich ascites-bearing mice to a greater extent than did a similar amount of free 5-FU.[1]

The present study was undertaken to investigate the antitumor effects of 5-FU entrapped in albumin microspheres after single-shot or multiple-shot administration, using Ehrlich ascites and solid tumors as model tumors *in vivo*.

## Experimental

**Materials**——5-Fluorouracil (5-FU) was obtained from Kyowa Hakko Co., Ltd. and bovine serum albumin (BSA) from Seikagaku Kogyo Co., Ltd. 5-Fluorouracil-6-$^3$H ($^3$H-5-FU) and $^{125}$I-human serum albumin ($^{125}$I-HSA) were purchased from the Japan Radioisotope Association. Pronase ($1.17\times10^6$ trypsin unit/g, Kaken Kagaku Co., Ltd.) was selected as a protease.

**Preparation of BSA Microspheres**——BSA microspheres containing 5-FU were prepared by a modification of the method of Scheffel *et al.*[6b] The drug content of the final product was 0.033 mg/mg.

***In Vitro* Drug Release**——Drug release from microspheres was determined by means of a dynamic dialysis system employing cellulose tubing. The procedure was described in detail elsewhere.[1] The effects of hydrolytic enzymes on the *in vitro* drug release were determined by adding protease (1 or 15 mg) or lysosomal fraction (1 mg) to the inner solution (pH 7.4 phosphate buffer containing 150 mg of microspheres) from the cellulose tubing. Lysosomal fraction was isolated from freshly collected Ehrlich ascites cells in 0.25 M sucrose solution by a modification of the method of Lewis *et al.*[10]

***In Vivo* Experiments**——Male ICR mice, weighing approximately 30 g, were used, and the dose of 5-FU was 0.5 mg/mouse.

In the first series of experiments, mice were intraperitoneally inoculated with $2\times10^7$ Ehrlich ascites cells. Five, 7 and 10 days after inoculation, the volume of the ascites and the number of tumor cells in the ascites were determined in mice treated with 0.25 ml of microsphere suspension, free 5-FU or 0.9% NaCl solution as a control 24 hr following tumor cell inoculation. Viable tumor cells in the ascites were counted microscopically with a Thoma counting chamber, with trypan blue staining. Furthermore, 0.25 ml of $^{125}$I-labelled albumin microsphere suspension containing nonlabeled 5-FU, nonlabeled albumin microsphere suspension containing $^3$H-5-FU or non-entrapped (free) $^3$H-5-FU solution was injected intraperitoneally 7 days after inoculation. Animals were sacrified at intervals and ascites fluid was collected to determine the 5-FU and microsphere levels in the ascites.

In the second series of experiments, 1, 5 and 9 days after intraperitoneal inoculation with $1\times10^6$ ascites cells of the tumor, the mice were injected intraperitoneally with 0.25 ml of microsphere suspension, free 5-FU or 0.9% NaCl solution. Changes in body weight and survival times of treated, tumor-bearing mice were recorded.

The third series of experiments was carried out to assess the antitumor effect of 5-FU given in microsphere form on Ehrlich solid tumor. A suspension of $2\times10^7$ ascites cells of the tumor was subcutaneously implanted into the scapular region of mice. Direct injection into the solid tumor of about 0.15 ml of 5-FU entrapped microsphere suspension, free 5-FU or 0.9% NaCl solution was done at 1 day for a single-shot administration or at 1, 5 and 9 days for multiple-shot administration after tumor implantation. Daily measurements of tumor size were taken with callipers. The maximal perpendicular dimensions (mm) of a tumor were averaged and the volume was evaluated on the base of a radial tumor cell distribution. Furthermore, the levels of 5-FU and microspheres in each tumor at various times following administration were determined. The procedures were as same in the first series of experiments on ascites cells.

**Analytical Methods**——To measure 5-FU and microsphere levels in the ascites or solid tumor, each tumor was collected. Each aliquot of tumor was mixed with the same amount of 0.9% NaCl solution, homogenized and centrifuged for 10 min to produce a supernatant. For measurement of tritium level, the supernatant was treated with a Protosol (New England Nuclear)-ethyl alcohol solution (1:2) and diluted directly with a scintillator (Aquasol II, New England Nuclear), then the radioactivity was determined with a liquid scintillation counter (LSC-651, Aloka Co.). For measurement of microsphere level, the radioactivity of $^{125}$I-labeled microspheres in the tumors was determined with an auto-gamma scintillation spectrometer (type 5110, Packard).

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## Results and Discussion

### (1) Effect of Hydrolytic Enzymes on the Release of 5-FU from Microspheres

The *in vitro* release of 5-FU from albumin microspheres continued for over one week, as shown in a preceding paper.[1] The effect of hydrolytic enzymes on the release of 5-FU from microspheres is shown in Fig. 1. The addition of lysosomal fraction to a microsphere suspension resulted in a small increase in the amount of drug release, and the addition of a protease (Pronase) resulted in a large increase. This increase may be due to digestion of albumin microspheres by the hydrolytic enzymes. This suggests that microspheres taken up into tumor cells by endocytosis could be digested by a lysosomal enzyme.

We have already suggested that 5-FU entrapped in albumin microspheres shows prolonged action against Ehrlich ascites carcinoma in mice,[1] and this may therefore be due to sustained release of 5-FU at the ascites and an increase in the intracellular concentration of the drug due to endocytosis.

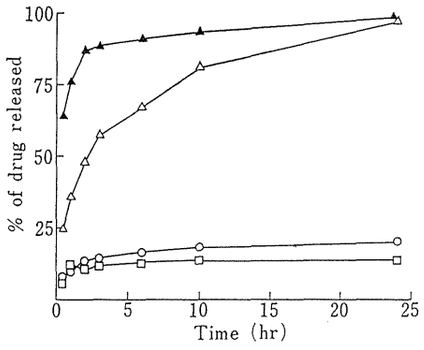


Fig. 1. Effect of Hydrolytic Enzymes on the Release of 5-FU from Albumin Microspheres

Treatment with 1 mg (△) or 15 mg (▲) of protease, 1 mg of lysosomal fraction (○) or buffer only (□). Each point represents the average of three experiments.

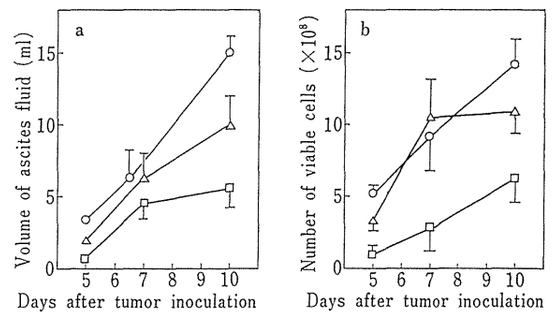


Fig. 2. Effects of Free and Albumin Microsphere-entrapped 5-FU on the Ascites Volume and Ehrlich Ascites Tumor Cell Count in Mice

Treatment with 0.9% NaCl solution (○), free 5-FU (△) and microsphere-entrapped 5-FU (□).
Results are expressed as the means ± S.E. of 5 mice.

### (2) Effect of Microsphere-entrapped 5-FU on the Growth of Ehrlich Ascites Cells

Upon intraperitoneal administration of free and microsphere-entrapped 5-FU to mice inoculated with Ehrlich ascites cells, the survival time of mice treated with the microsphere-entrapped drug is longer.[1] In this work, we studied the effect of microsphere-entrapped 5-FU on the production of ascites fluid and cell counts to confirm the therapeutic effect of the drug entrapped in albumin microspheres. The elimination rates of free and microsphere-entrapped 5-FU from the injection site, and the biodegradation of albumin microspheres after injection were also estimated.

Following inoculation, the viable cell counts and the ascites volume increased proportionally (Fig. 2). The viable cell counts after treatment with microspheres were significantly different from that for 0.9% NaCl solution (Student's t-test: 5 days, $p<0.001$; 7 days, $p<0.05$; 10 days, $p<0.02$). However, a group treated with free 5-FU was not different from NaCl solution alone (5 days, $p<0.01$; 7 and 10 days, no significance). These results suggest that, in the

treatment of tumor-bearing mice, 5-FU entrapped in microspheres may, under certain conditions, be superior to non-entrapped 5-FU because of the sustained release of 5-FU from the albumin microspheres.

The time course of microsphere and 5-FU levels in the ascites are presented in Fig. 3, which shows the elimination of radioactivity due to $^{125}$I-labeled microspheres and $^{3}$H-5-FU in the ascites. The metabolism and elimination of non-entrapped 5-FU were fast, and the percentage of tritium radioactivity remaining at 24 hr after injection was only 0.93% of the dose. The percentage of tritium radioactivity released from microspheres and remaining in the ascites was 2.46% at 24 hr and 1.31% at 48 hr after injection of microsphere-entrapped 5-FU. In contrast, the loss of albumin microspheres from the injection site at 24 hr after injection was extremely small. These data suggest that the biodegradation of albumin microspheres in the ascites is very slow. 5-FU which is slowly released from the microspheres is more effective against the Ehrlich ascites cells; the rate of multiplication of Ehrlich ascites cells was clearly lower in the presence of microsphere-entrapped 5-FU than non-entrapped 5-FU.

### (3) Effect of Multiple-shot Administration of Microsphere-entrapped 5-FU on Ehrlich Ascites Carcinoma

When microsphere-entrapped 5-FU was intraperitoneally injected 24 hr after inoculation with Ehrlich ascites cells, the life span was increased by 20.5% compared with a control (0.9% NaCl solution).[1)] In the above experiments, however, suppression of tumor growth ceased after one week, presumably because the amount of 5-FU delivered by single-shot administration of microspheres was therapeutically insufficient by one week after administration. We therefore examined the effects of multiple-shot administration of microsphere-entrapped 5-FU on the tumor growth. Figs. 4 and 5 show the changes in body weight and survival of mice after injection with the microspheres 1, 5 and 9 days after inoculation with Ehrlich ascites carcinoma. The increase in body weight after administration of the microspheres was significantly smaller than that in another group receiving 0.9% NaCl solution or free 5-FU.[11)] The

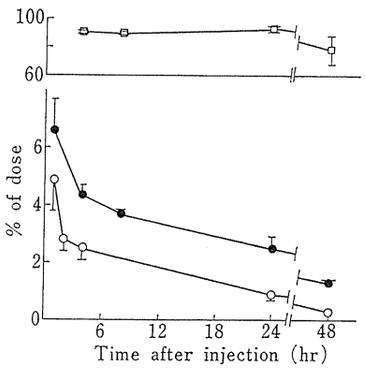


Fig. 3. 5-FU and Albumin Microsphere Levels in Ascites Fluid after Administration of Free or Microsphere-entrapped 5-FU

Free 5-FU (○), 5-FU released from microspheres (●), microsphere level (□).
Results are expressed as the means±S.E. of 3 mice.

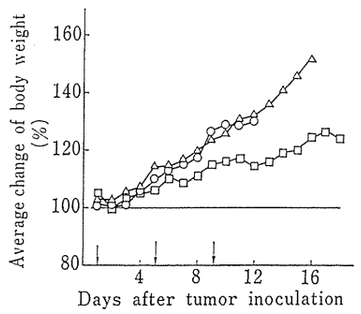


Fig. 4. Change in Body Weight of Ehrlich Ascites Carcinoma-bearing Mice after Multiple-shot Administration of Free or Albumin Microsphere-entrapped 5-FU

Treatment with 0.9% NaCl solution (○), free 5-FU (△) and microsphere-entrapped 5-FU (□).
Each point represents the average of 10 mice.
Arrows indicate the administration of free or albumin microsphere-entrapped 5-FU.

11) Microspheres/0.9% NaCl solution, $p<0.05$, 9 days following inoculation; microspheres/free 5-FU, $p<0.05$, 12 days following inoculation.



effect of multiple-shot administration of microsphere-entrapped 5-FU was superior to that of multiple-shot administration of free 5-FU. Survival time was also improved from 20.5% for single-shot administration of the microspheres to 52.0% for multiple-shot administration. These results indicate that multiple-shot administration of albumin microsphere-entrapped 5-FU may be effective in cancer chemotherapy.

### (4) Effect of Single-shot or Multiple-shot Administration of Microsphere-entrapped 5-FU on Ehrlich Solid Tumor

The effect of entrapped 5-FU on Ehrlich solid tumor was studied. Fig. 6 shows 5-FU levels and radioactivity due to $^{125}$I-labeled microspheres in the solid tumors after injection of free or microsphere-entrapped 5-FU into solid tumor-bearing mice. Solid tumor cells are not mobile and the neoplasmic tissue is rigid, so the disappearance of 5-FU from the solid tumor should be slower than that from the ascites. In the solid form, the elimination of tritium radioactivity of 5-FU was apparently retarded by slow release of 5-FU from albumin microspheres compared with that of free 5-FU. The results indicate the utility of intratumoral injection of the microspheres in the therapy of solid tumors.

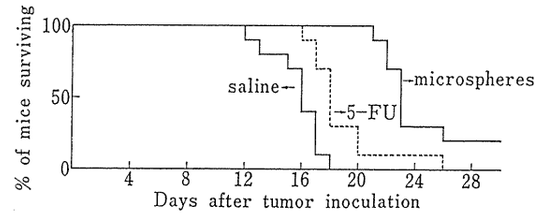


Fig. 5. Effect of Multiple-shot Administration of Free or Albumin Microsphere-entrapped 5-FU on the Survival of Mice Inoculated with Ehrlich Ascites Carcinoma

Two out of 10 mice given albumin microsphere-entrapped 5-FU survived over 60 days.

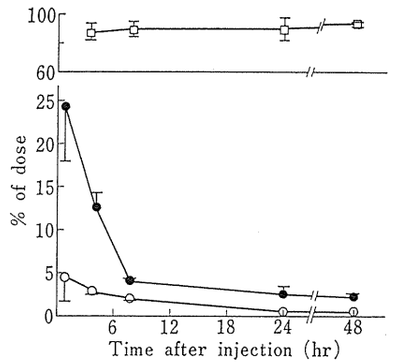


Fig. 6. 5-FU and Albumin Microsphere Levels in the Tumor after Administration of Free or Albumin Microsphere-entrapped 5-FU

Symbols: as in Fig. 3.
Results are expressed as the means±S.E. of 3 mice.

Fig. 7 shows the effects of free and microsphere-entrapped 5-FU on the growth of Ehrlich solid tumor. Nearly all mice in the groups administered 0.9% NaCl solution or free 5-FU had gross solid tumor involvement. Microsphere-entrapped 5-FU, however, was rather active against the solid tumor, and the tumor size became about 1/5 at 10 days and about 1/10 at 20 days after tumor inoculation compared with other groups receiving 0.9% NaCl solution or free 5-FU. This suppression of tumor growth at the inoculation site may be dependent on the prolonged release of 5-FU from the albumin microspheres.

Furthermore, we studied the effect of multiple-shot administration of microsphere-entrapped 5-FU on the Ehrlich solid tumor. Marked suppression of tumor growth at the inoculation site was observed on administering microsphere-entrapped 5-FU. This antitumor activity was surprisingly strong compared with single-shot administration of microsphere-entrapped 5-FU to the solid tumor (Fig. 8).

The experimental results in this paper show the antitumor effect of albumin microsphere-entrapped 5-FU to be greater than that of free 5-FU in two tumor systems, Ehrlich ascites

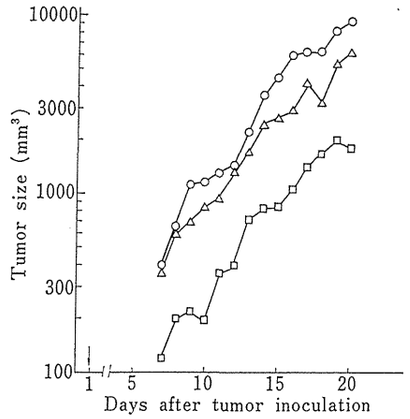


Fig. 7. Effect of Single-shot Administration of Free or Microsphere-entrapped 5-FU on the Growth of Ehrlich Solid Tumor

Symbols: as in Fig. 4.
Each point represents the average of 10 mice.
The arrow indicates the administration of free or albumin microsphere-entrapped 5-FU.

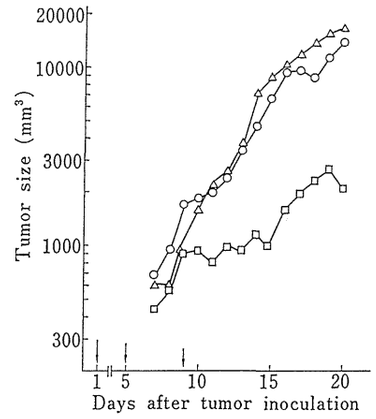


Fig. 8. Effect of Multiple-shot Administration of Free or Microsphere-entrapped 5-FU on the Growth of Ehrlich Solid Tumor

Symbols: as in Fig. 4.
Each point represents the average of 10 mice.
Arrows indicate the administration of free or albumin microsphere-entrapped 5-FU.

carcinoma and solid tumor. Further, treatment by multiple-shot administration of microsphere-entrapped 5-FU was particularly effective.

This result may be explained in terms of the sustained release of 5-FU from albumin microspheres *in vivo*. 5-FU has been used clinically for the treatment of various tumors by parenteral administration,[12] because oral administration of this drug shows higher toxicity than parenteral administration.[13] It seems likely that 5-FU entrapped in albumin microspheres shows pronounced antitumor activity and low toxicity.

Studies are presently under way in our laboratory to examine the site specificity of the drug and its prolonged action against experimental liver tumor after intravenous injection of albumin microsphere-entrapped 5-FU.

**Acknowledgement** The authors are grateful to Miss M. Igarashi for her help in some experiments.

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[Chem. Pharm. Bull. 29(5)1433—1438(1981)]

# Drug-carrier Property of Albumin Microspheres in Chemotherapy. V.[1] Antitumor Effect of Microsphere-entrapped Adriamycin on Liver Metastasis of AH 7974 Cells in Rats

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Bovine serum albumin microspheres containing adriamycin were prepared by heat solidification of albumin in albumin-adriamycin aqueous solution in cottonseed oil emulsions. The efficiency of the microspheres as a drug carrier of adriamycin was evaluated in liver metastasis caused by the injection of AH 7974 tumor cells into the portal vein of rats as a model of release of such cells into the vein during the course of surgical removal of gastric cancer. Adriamycin entrapped in the microspheres exhibited sustained *in vitro* release which followed first-order kinetics. After intraportal injection in rats, the microspheres and entrapped agent distributed mainly in the liver, and the disappearance rate from the tissue was very slow in comparison with that of free drug. The survival times of rats bearing AH 7974 liver metastasis were prolonged by intraportal administration of microspheres containing adriamycin. In contrast, free adriamycin or microspheres without entrapped drug did not significantly increase the life span over the control. These results suggest that albumin microspheres containing adriamycin may be applicable as a drug carrier in the adjuvant chemotherapy of liver metastasis.

**Keywords**——albumin microsphere; drug carrier; adriamycin; sustained release; preferential distribution; AH 7974 liver metastasis; antitumor effect; adjuvant chemotherapy

One of the goals in cancer chemotherapy is directing drugs selectively into tumor tissues to minimize the side effects on normal tissues. In the past, considerable efforts have been directed toward the development of drug carriers, *i.e.* DNA complexes,[2] liposomes,[3] and emulsions,[4] able to deliver antitumor agents selectively into tumor tissues. We have been studying the utility of albumin microspheres containing 5-fluorouracil as a delivery system, in the hope of enhancing the drug accumulation in the liver of mice after intravenous injection[5,6] and obtaining prolonged action against Ehrlich ascites and solid tumors.[1,7] This series of studies was undertaken to investigate the ability of antitumor agents entrapped in albumin microspheres to prevent liver angiosarcoma or liver metastasis caused by the release of tumor cells through the portal vein during the course of surgical removal of gastric cancer.

The present investigation demonstrates the antitumor effect of adriamycin-containing microspheres on the liver metastasis in rats which had been injected with AH 7974 cells into the portal vein.

## Experimental

**Materials**——Non-labeled and $^{3}H(G)$-labeled adriamycin were from Kyowa Hakko Co., Ltd. Bovine serum albumin (BSA) (Fr. V. Powder) was purchased from Seikagaku Kogyo Co., Ltd. and $^{125}I$-human serum albumin from Japan Radioisotope Association. Cottonseed oil was selected as a vegetable oil. Nonionic surfactants, Span 85 and polysorbate 80 were obtained from Wako Pure Chemicals. All other chemicals were commercial reagent-grade products.

**Animals**——Male Donryu rats, weighing about 150 g, were used in all animal experiments.

**Preparation of BSA Microspheres**——The basic procedure of Scheffel *et al.*[8] was modified for the preparation of albumin microspheres containing adriamycin. A mixed aqueous solution containing 100 mg of adriamycin and 250 mg of BSA in a volume of 4 ml was emulsified into 100 ml of cottonseed oil containing 10% (v/v) Span 85 by the method described in a previous paper.[6] The solidification temperature of albumin

in oil emulsions was 170—180°. The average diameter and drug content of the final product were about 1.44 μm and 0.15 mg/mg.

***In Vitro* Drug Release**——Adriamycin release from microspheres was determined by means of a dynamic dialysis system employing cellulose tubing. The procedure was described elsewhere.[7] Adriamycin was determined by fluorescence intensity measurement at 470 nm (excitation) and 560 nm (emission)[9] in a Hitachi 650-60s fluorescence spectrophotometer.

**Procedure for Preparing Liver Metastasis**——AH 7974 cells were maintained by weekly transplantation in ascites form in rats. For preparing liver metastasis, a rat was anesthetized by intraperitoneal injection of sodium pentobarbital and the portal vein was exposed by a midline abdominal incision. AH 7974 ascites cells ($10^6$ cells/0.1 ml) were implanted into the portal vein, and the bleeding after administration was stopped by finger pressure. The abdominal cut was sewn up, and chloramphenicol ointment (Sankyo Co., Ltd.) was applied to prevent infections.

**Microsphere and Adriamycin Distributions in Rats**——$^{125}$I-labeled microspheres containing non-labeled adriamycin or non-labeled microspheres containing $^3$H(G)-labeled adriamycin (2 mg of microspheres containing 300 μg of adriamycin) suspended in 0.9% NaCl solution containing 0.2% (v/v) polysorbate 80, or 300 μg of free $^3$H(G)-adriamycin in 0.9% NaCl solution was injected into the portial vein or tail vein in rats. At 10 min, 1 hr, and 1 day after administration, 1 ml of blood was taken by heart puncture and each rat was sacrificed by decapitation. Several tissues (heart, lung, spleen, kidney, liver, and intestine) were removed and weighed wet. $^{125}$I-labeled microspheres in blood and isolated tissues were determined with a Packard 5110 Auto-gamma scintillation spectrometer. Tritium levels in blood and isolated tissues were measured with an Aloka LSC-651 liquid scintillation spectrometer after combustion in an Aloka ASC-111 sample oxidizer.

The amount of tritium released from the microspheres in the liver (*i.e.*, total tritium in the liver minus microsphere-entrapped tritium) was determined as follows. The liver was homogenized with 3 volumes of 0.9% NaCl solution. Two ml of homogenate was used for each determination. To each sample, 1 ml of 16.5% $AgNO_3$ solution was added as a deproteinization reagent. The tubes were shaken vigorously for 20 min then centrifuged at 1500 rpm for 10 min, and the supernatant was subjected to liquid scintillation counting after combustion in a sample oxidizer.

Quenching correction was done by the external standard ratio method for tritium radioactivity. The tissue distribution of radioactivity is represented as % of administered dose in whole tissue (blood) or per gram of tissue (ml of blood).

**Effect of Drug-containing Microspheres on Liver Metastasis**——The antitumor effect of free or entrapped adriamycin against AH 7974 liver metastasis was studied in terms of animal survival (10 mice per group). AH 7974 cells alone were administered into the portal vein in rats of the control group. Three hundred μg of adriamycin or 2 mg of microspheres containing 300 μg of adriamycin was co-administered with AH 7974 cells in the treated groups, and 2 mg of microspheres without entrapped drug was administered to 5 rats to check the effect of the microspheres themselves on the tumor.

The animals were housed in standard rat cages and observed for 60 days. The median survival time of treated rats against that of control rats (T/C%) was used as a measure of antitumor effect.

## Results

Figure 1 shows the *in vitro* release of adriamycin from albumin microspheres. It is clear that, in contrast to the rapid release of free adriamycin from a Visking dialysis sac, the release of adriamycin from microspheres and through the Visking sac was small. The half-release time of the free drug through the Visking sac, $T_{50\%}$, was about 0.5 hr. The release of adriamycin from microspheres followed first-order kinetics for about four days after the start of the experiment. The half-release time of adriamycin from microspheres was about 7.3 days and the cumulative amount released during 7 days was 42.5%. Entrapment in microspheres resulted in a remarkable retardation of the release of adriamycin.

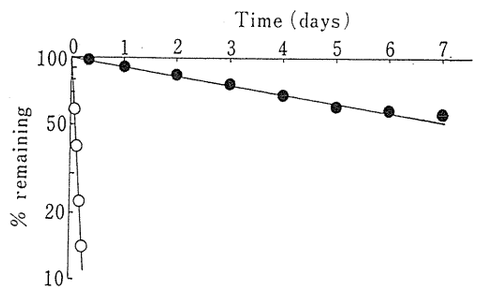


Fig. 1. Release of Adriamycin from a Dialysis Sac

○; free adriamycin,
●; microspheres containing adriamycin.

Figure 2 shows the distribution of $^3$H(G)-adriamycin to various organs at 10 min, 1 hr, and 1 day after intravenous or intraportal injection. When the route of administration was changed from the tail vein to the portal vein, the distribution of adriamycin in the liver at 10 min after injection increased from 3.9%/g liver to 6.3%/g. Although the data are not shown, the blood concentration after intraportal injection was lower than that in all tissues measured in this experiments. After intraportal injection, the drug levels in the heart and kidney, which are adversely affected by adriamycin, were lower than those after intravenous injection.



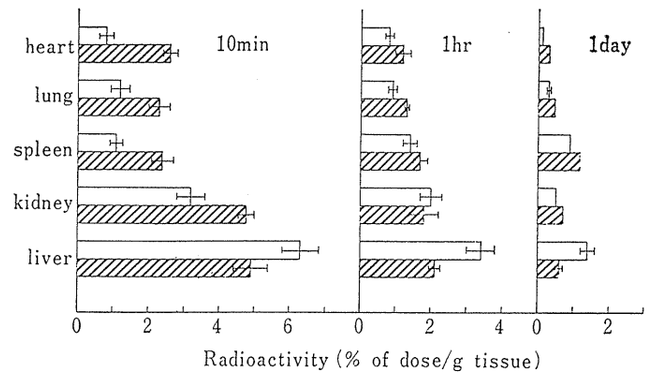


Fig. 2. Tissue Distributions at 10 min, 1 hr, and 1 day after Injection of Free $^3$H(G)-Adriamycin

▨; intravenous injection,
□; intraportal injection.
Each column represents the mean value of 4—7 rats. Vertical bars indicate S.E.M.

Figure 3 shows the distribution of $^{125}$I- and $^3$H-radioactivities after intraportal injection of $^{125}$I-labeled microspheres containing non-labeled adriamycin or non-labeled microspheres containing $^3$H(G)-adriamycin. As $^{125}$I-radioactivity is rigidly bound to the microspheres,[10] the distribution of $^{125}$I-radioactivity corresponds to the microsphere distribution in rats. Since adriamycin is little metabolized,[11] the distribution of adriamycin entrapped in microspheres and released from microspheres. These were named microsphere fraction (M) and free fraction (F), respectively. At 10 min, 1 hr, and 1 day after intraportal injection of $^{125}$I-microspheres, the percentages of dose per gram of liver were 13.9, 13.9, and 13.4, respectively. $^{125}$I-radioactivity in other tissues and blood amounted to only a trace. After intraportal injection of microspheres containing $^3$H(G)-adriamycin, tritium radioactivity was mainly distributed in the liver. However, the percentage of dose in the liver after administration of microspheres containing $^3$H(G)-adriamycin was smaller than that after injection of $^{125}$I-microspheres. The difference might result from release of adriamycin from microspheres in the blood and liver and rigid binding of $^{125}$I-radioactivity. The adriamycin released might distribute to other tissues. This view was supported by the finding that tritium levels (%) in tissues other than the liver were higher than $^{125}$I-levels (%), as shown in Fig. 3. At 10 min, 1 hr, and 1 day after injection of microspheres containing $^3$H(G)-adriamycin, the tritium levels of the free fraction of adriamycin were 1.21, 0.90, and 0.37% per gram of liver, and the free fractions were 1/10, 1/13, and 1/32, respectively.

Figure 4 shows the total amount of radioactivity in the liver (% of dose) at 10 min, 1 hr, and 1 day after intraportal injection of free of microsphere-entrapped $^3$H(G)-adriamycin, or $^{125}$I-microspheres, as well as the total amount of radioactivity in the liver after intravenous injection of free $^3$H(G)-adriamycin or $^{125}$I-microspheres. Adriamycin in the liver was almost eliminated at 1 day after intravenous or intraportal injection of the free drug. In contrast,

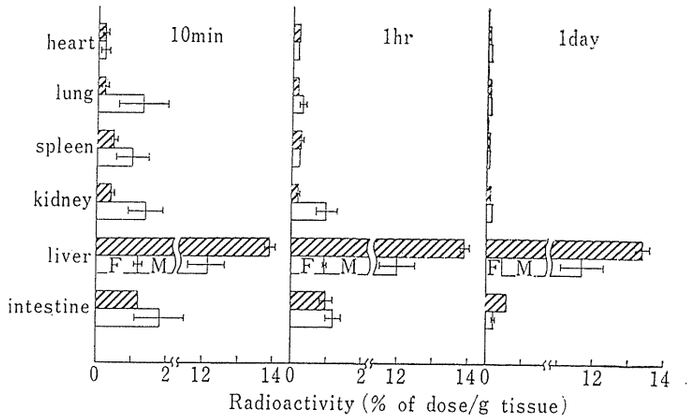


Fig. 3. Tissue Distributions of $^{125}$I- and $^{3}$H-Radioactivities at 10 min, 1 hr, and 1 day after Intraportal Injection of $^{125}$I-Microspheres or Non-labeled Microspheres containing $^{3}$H(G)-Adriamycin

▨; $^{125}$I-microspheres,
□; microspheres containing $^{3}$H(G)-adriamycin, F and M; free and microsphere fraction (see the text).
Each column represents the mean value of 4—5 rats. Vertical bars indicate S.E.M.

$^{3}$H- and $^{125}$I-radioactivities even at 1 day after intraportal injection of microspheres were very high. At 10 min after intravenous injection of $^{125}$I-microspheres, 85.3% of the dose was incorporated in the whole liver, and even at 1 day, 79.7% of the microspheres remained in the liver. These results indicate that the microspheres preferentially distribute into the liver, and that a high level is maintained in the liver. Radioactivity in the liver after intraportal injection of $^{125}$I-microsphers was slightly higher than that after intravenous injection. This result might be explained by the difference in the amount of microspheres first-passed in the liver.

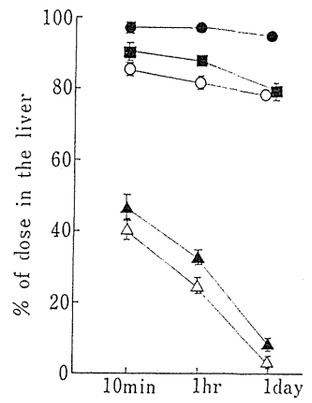


Fig. 4. Tissue Distributions of $^{125}$I- and $^{3}$H-Radioactivities in the Liver after Injection of Microspheres or Adriamycin

○; $^{125}$I-microspheres after intravenous injection,
●; $^{125}$I-microspheres after intraportal injection,
■; microspheres containing $^{3}$H(G)-adriamycin after intraportal injection,
△; free $^{3}$H(G)-adriamycin after intravenous injection,
▲; free $^{3}$H(G)-adriamycin after intraportal injection.
Each point represents the mean value of 4—7 rats.
Vertical bars indicate S.E.M.

Figure 5 and Table I compare the antitumor effects of free and microsphere-entrapped adriamycin, and microspheres without entrapped drug against AH 7974 liver metastasis. When $10^{6}$ cells of AH 7974 ascites were inoculated into the portal vein in rats, the animals died between 10 and 16 days later due to the metastasis (control in Fig. 5), and the median

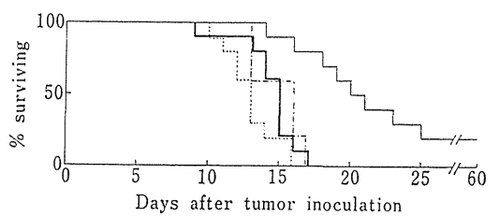


Fig. 5. Effect of Intraportally Administered Adriamycin or Microspheres on AH 7974 Liver Metastasis in Rats

--------; control,
———; free adriamycin,
———; microspheres containing adriamycin,
—·—·—; microspheres without drug.



Table I. Effect of Intraportally Administered Adriamycin or Microspheres on AH 7974 Liver Metastasis in Rats

| | Survival[a] days | T/C % | Number of rats survived/treated |
|---|---|---|---|
| Control | 13.0±0.6 | — | 0/10 |
| Free adriamycin | 14.0±0.7 | 110 | 0/10 |
| Microspheres containing adriamycin | 19.5±1.3[b] (27.6±5.3)[c] | 150[b] (212)[c] | 2/10 |
| Microspheres without drug | 15.0±0.8 | 115 | 0/5 |

*a*) Mean value±S.E.M.
*b*) Calculated for 8 rats that survived for less than 60 days.
*c*) Calculated for 10 rats.

survival time was 13.0 days. Injection of free adriamycin or microspheres without entrapped drug did not give any significant difference in survival from the control. In contrast, 2 out of 10 rats survived over 60 days after administration of microspheres containing adriamycin; the median survival time of the other 8 rats was 19.5 days and T/C % was 150.

## Discussion

The development of a suitable method for the preparation of drug-containing albumin microspheres requires a knowledge of the physicochemical properties of the agents. The stability of drug-albumin aqueous solution in cottonseed oil emulsions and the particle size of albumin microspheres might be influenced by the viscosity of the mixed aqueous solution at the first step of the preparation of microspheres. The adriamycin content in the present microspheres was 0.15 mg/mg, which was higher than the 5-fluorouracil content (0.033 mg/mg) in microspheres reported elsewhere.[1,7] The difference of drug contents in microspheres might be due to a difference of drug leakage from microspheres at the washing stage of microspheres in the preparation, and this in turn might depend on differences of physicochemical interactions between albumin and drug, and/or change of the molecular mobility in the microspheres due to the difference of molecular size (130.08 molecular weight for 5-fluorouracil and 579.98 for adriamycin). As shown in Figure 1, the microspheres containing adriamycin exhibited sustained drug release. Since the burst effect[12] as observed in the case of *in vitro* 5-fluorouracil release from microspheres[7] was not observed in the case of adriamycin release, the efficiency of adriamycin entrapment in the microspheres appears to be higher than that of 5-fluorouracil. The release mechanism of drugs from microspheres is very complex, and further study is needed in order to understand the release characteristics of albumin microspheres containing various chemotherapeutic agents. We plan to study the effect of solidification temperature during preparation on the *in vitro* release of adriamycin.

After intravenous or intraportal injection, albumin microspheres were mainly distributed in the liver (Figs. 3 and 4). Microspheres after intravenous injection also distributed in the lung, but this was not the case after intraportal injection. If the drug or microspheres themselves caused side effects, especially in the lung, intraportal injection would be better than intravenous injection. Further if tumor cells and microspheres were released through the same route, they might disperse to the same sites in the body. Therefore, intraportal injection might be more useful than intravenous input against metastasis from gastric cancer to the liver through the portal vein.

From the results of Figure 5 and Table I, adriamycin entrapped in the microspheres shows a greater effect on liver metastasis than free adriamycin or non-entrapped microspheres, or the control. T/C % for rats treated with microspheres without entrapped drug was 115, suggesting that the microspheres physically interacted the tumor cells. The number of the tumor cells

inoculated in rats in this experiments was $10^6$ cells per rat, whereas the amount of microspheres was 2 mg weight ($2\times10^8$ particles).[13] Thus, a metastatic tumor cell might be surrounded by scores of microspheres. The microspheres which were approaching the tumor cell might be phagocytized in the cell, or might release the entrapped adriamycin. Since microspheres containing adriamycin show sustained release (Fig. 1), an effective level of adriamycin may be maintained around the tumor cell.

In conclusion, the present results that albumin microspheres show sustained release of adriamycin, preferential distribution in the liver after intraportal injection, and preventive effects on AH 7974 liver metastasis demonstrate the superiority of intraportal injection of microspheres in adjuvant chemotherapy of liver metastasis. It should be possible to improve the efficacy of antitumor agents entrapped in microspheres by the improvement of the *in vitro* drug release characteristics.

**Acknowledgement** The authors thank Dr. Hiroshi Sato, Sasaki Institute, for valuable suggestions and for providing AH 7974 cells.

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# BIOMEDICAL APPLICATIONS OF MAGNETIC FLUIDS. I. MAGNETIC GUIDANCE OF FERRO-COLLOID-ENTRAPPED ALBUMIN MICROSPHERE FOR SITE SPECIFIC DRUG DELIVERY *IN VIVO*

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Magnetic guidance of magnetic albumin microsphere for site specific drug delivery was investigated *in vivo*. After intravenous injection in mice, magnetic microspheres localized in the site (lung) at which two permanent magnets were placed. Injection into the renal artery in rats also indicated that the microspheres were concentrated at the kidney by a magnetic field. When magnet was not applied, however, the microspheres were concentrated mainly in the liver. Such preferential localization by magnetic means suggested that magnetic albumin microspheres could become effective drug carriers with site specificity for the delivery of chemotherapeutic agents in cancer therapy.

**Keywords**—magnetic albumin microsphere; magnetic fluid; drug carrier; site specificity; drug delivery; magnetic guidance



Localization of chemotherapeutic agents to specific sites would reduce the systemic dose of a given drug while still achieving effective local concentration of the drug. One chemical approach to targeting chemotherapeutic agents is inclusion of the agents within carriers. Recent examples include entrappment of daunomycin into DNA[1] and other antitumor agents into liposomes.[2,3] We recently reported that 5-fluorouracil entrapped in albumin microspheres was present in high levels in the liver of mice after intravenous injection,[4,5] and suggested sustained release and prolonged action of entrapped drug occurred in Ehrlich ascites[6] and solid carcinoma.[7] However, intravenous injection of such liposomes and microspheres results in their uptake predominantly by the reticuloendothelial system,[4,5,8] especially the Kupffer cells in the liver.[9] More recently, Widder *et al.*[10] suggested that since magnetic microspheres injected into the ventral caudal artery could be localized to some extent to a predetermined tail segment by an externally applied magnetic field, magnetically guided albumin microspheres entrapped drugs would be useful as a drug delivery system with site specificity.

The experimental developments in this approach have been prevented by two difficulties, *i.e.* the control of a magnetic field at a target topical site and the preparation of an active drug with a magnetically responsive character. If site specific drug delivery of antitumor agents could be achieved with magnetic means, this delivery system may eliminate adverse side effects that are often the sequelae of generated systemic drug distribution. This paper describes the utility of magnetic albumin microsphere as a drug carrier with target specificity by measuring microsphere levels in the lung and kidney after intravenous and intra-renal-arterial administration, respectively.

Magnetic albumin microspheres were prepared with bovine serum albumin, $^{125}$I-human serum albumin (50 $\mu$Ci/ml, Japan Radioisotope Association) and magnetic fluid* (Type W-35, Taiho Industries Co., Ltd.) by a modification of the method of Widder *et al.*[11] The final microspheres contain about 50% magnetite ($Fe_3O_4$). Two types of microspheres, namely small microsphere (1 $\mu$m in average diameter) and large microsphere (3 $\mu$m), were used in the experiments.

* Average diameter of the colloidal particles is 100—200 A.



In the first experiments, the mouse lung was selected as a model for *in vivo* testing of microspheres for two reasons. (i) The lung tumor occupies high ratios in many malignancies. (ii) The microspheres after intravenous injection pass through the lung until sequestration in the liver. One mg of small or large microsphere was injected into the tail vein in ICR mice, weighing about 30 g, as 0.2 ml of a suspension. Two magnets (Super Disc Magnet, No. 30730, Edmund Scientific Co.) with a magnetic introduction of about 3000 Gauss were placed on the breast and back of mice throughout the experiment so as to concentrate the microsphere into the lung. Mice were killed 10 min after administration, and $^{125}$I-labeled microspheres in various tissues were determined by an Auto-gamma scintillation spectrometer (Type 5110, Packard).

The rat kidney was selected for a model in the second experiments. It is difficult to collect the microspheres in other tissues except the lung or heart after intravenous injection, then the route of the administration was changed to intra-artery. Administration of the microspheres through the renal artery was carried out by cannulating a polyethylene tubing and forming a new vessel bypassing the original artery, because the polyethylene tubing is easy for injection. One mg of small microsphere was injected into the left renal artery in male Wistar rats weighing about 220 g. Two magnets were directly placed throughout the experiments at the both sides of left kidney which was exposed by a midline abdominal incision. Distribution of microsphere 10 and 60 min after administration was examined by the method described previously. The distribution of $^{125}$I-albumin microspheres to various organs is represented as % of dose per gram tissue or that in whole tissue.

The experimental results in mice were shown in Fig. 1. After intravenous injection of small microspheres in mice without magnet (control), about 3.9% of the administered dose (15.8%/g tissue at the concentration) was found in the lung. When the particle size of the microspheres was enlarged from 1 to 3 $\mu$m in diameter, uptake of microsphere in the lung increased from 3.9% of dose (15.8%/g tissue) to 10.7% (51.7%/g). This results may indicate, considering that blood capillary is narrow, that the large microspheres lead to the occlusion of the capillary. When two magnets were applied to the lung, however, the microsphere level in the lung increased about four-fold for small microsphere and twice for large microsphere compared with each control. After injection of large microsphere, a peak lung

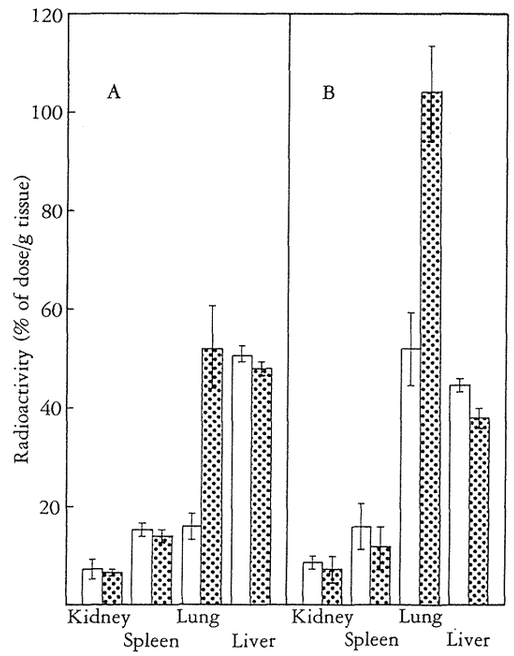


FIG. 1. *Tissue Distribution of Radioactivity at 10 min after Intravenous Injection of Magnetic Microsphere (1 and 3 $\mu$m in diameter)*

*A; 1 $\mu$m in average diameter, B; 3 $\mu$m in average diameter.* ☐ *; control (no magnet),* ▨ *; treatment with two magnets throughout the experiments. Each column represents the mean value of 3—5 experiments. Vertical bars indicate S.E.M.*

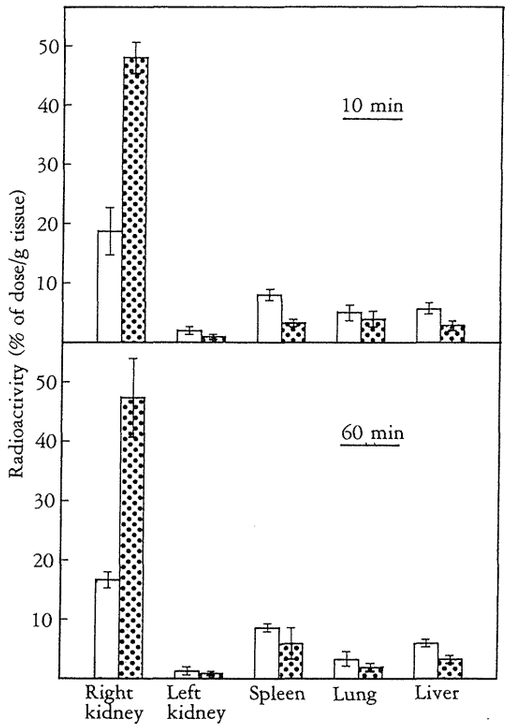


FIG. 2. *Tissue Distribution of Radioactivity at 10 and 60 min after Administration into the Left Renal Artery of Magnetic Microsphere (1 μm in diameter)* ▭ *; control (no magnet),* ▩ *; treatment with two magnets throughout the experiments. Each column represents the mean value of 3 experiments. Vertical bars indicate S.E.M.*

level (104.0%/g tissue) was measured, but the amount of the microspheres in the lung was only 21.6% of the dose. When the strength of magnetic field is increased, this value (21.6%) might be improved considering that Widder *et al.*[10] used high magnetic field strength (8000 oersteds) to retain 37—65% microspheres into the tail after infusion through the ventral caudal artery.

After intravenous injection, the microspheres did not concentrate in the kidney and localized mainly in the liver as mentioned above.* Though the data are not illustrated, intravenous injection in rats applied two magnets at the kidney did not increase in the microsphere level in the magnet site.** Microsphere level in the kidney after intra-renal-arterial administration (Fig. 2, control) was higher than that in the experiments of intravenous injection with and without magnets. Administration into the rat kidney with magnets, on the other hand, concentrated about 56% of the dose (48%/g tissue) in the kidney at 10 min as shown in Fig. 2 (treatment with two magnets), and the value was about 2.5-fold higher than that in the control. The microsphere level in the kidney at 60 min was not different from that at 10 min, which suggested that the microspheres in the kidney were retained by the magnets.

A carrier system for the site specificity of chemotherapic agents by magnetic means is a unique idea and magnetic albumin microspheres may provide a new means of treatment in cancer chemotherapy. Presently, we plan to investigate the applicability of such drug carriers to several experimental tumor systems.

* After intravenous injection of the microsphere without magnets, the level in the kidney was 1.5% of the dose (1.2%/g tissue).

** After intravenous injection with magnets, the level in the kidney was 1.7% of the dose (1.4%/g tissue).



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# Lag Time involved in the Experiments on Drug Release from Ointments[1]

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Explicit expression was derived for the lag time due to drug accumulation in the diffusion layer of the sink solution, which is encountered at experiments on drug release from ointments.

Higuchi's equation (Eq. 1) that expresses the amount ($Q$) of drug released from the ointment, in which the initial drug concentration is $C_0$, into a perfect sink during time $t$, indicates that the plots of $Q$ against square root of $t$ give a straight line through the origin.

$$Q = 2C_0\sqrt{\frac{Dt}{\pi}} \qquad \text{Eq. 1}$$

In practice, the straight line does not always pass the origin but shows some time lag.[3] One reason for this is explained by the well known lag time of Barrer,[4] which is a measure of the period required for the accumulation of the drug in the membrane, if the membrane is used, (and in the diffusion layer of the sink solution at the neighbor of the ointment). If the drug concentration in the ointment is constant, Barrer's lag time is given by

$$\tau_B = \frac{h^2}{6D} \qquad \text{Eq. 2}$$

were $h$ is the thickness of the membrane and $D$ is diffusion constant. In the case of drug release experiments, however, drug concentration in the ointment is not constant but decreases every minute.

Aim of this note is to express theoretically the lag time observed under such experimental situations.

## Theoretical

Let $D_1$ be the diffusion constant in the diffusion layer, the thickness of which is $h$, and $D_0$ that in the ointment. Also, let $C_1$ be the concentration in the diffusion layer and $C_0$ that in the ointment (Fig. 1). Then assume that the diffusion is expressed by the following equations:

$$\text{at } t=0 \quad C_1 = 0 \quad (-h \leqq x < 0) \qquad \text{Eq. 3}$$

$$C_0 = C_{in} \quad (0 \leqq x) \qquad \text{Eq. 4}$$

$$\text{at } t>0 \quad \frac{dC_1}{dt} = D_1\frac{d^2C_1}{dx^2} \quad (-h < x < 0) \qquad \text{Eq. 5}$$

$$\frac{dC_0}{dt} = D_0\frac{d^2C_0}{dx^2} \quad (0 < x) \qquad \text{Eq. 6}$$

1) Partly presented at the 95th Annual Meeting of Pharmaceutical Society of Japan, Nishinomiya, April 1975.
2) Location: *3190, Gofuku, Toyama, 930, Japan.*
3) K. Kakemi, H. Kameda, M. Kakemi, M. Ueda, and T. Koizumi, *Chem. Pharm. Bull.* (Tokyo), 23, 2114 (1975), cf. Fig. 3.
4) R.M. Barrer, "Diffusion in and through Solids," Cambridge University Press, Cambridge, England, 1951, p. 477.



$$D_1\frac{dC_1}{dx} = D_0\frac{dC_0}{dx} \quad (x=0) \qquad \text{Eq. 7}$$

$$C_0 = pC_1 \quad (x=0) \qquad \text{Eq. 8}$$

$$C_1 = 0 \quad (x=-h) \qquad \text{Eq. 9}$$

$$Q = D_1\int_0^t \left(\frac{dC_1}{dx}\right)_{x=-h} dt \qquad \text{Eq. 10}$$

where $p$ is the partition coefficient of the drug at the interface ($x=0$).

Solution to these equations with the indicated boundary conditions are obtained by means of the method given by Carslaw and Jaeger.[5] The amount of drug released into sink, $Q$, is expressed by Eq. 11.

$$Q = \frac{4C_{in}}{p+\sqrt{\frac{D_1}{D_0}}}\sqrt{\frac{D_1t}{\pi}}\cdot\sum_{n=0}^{\infty}\left(\frac{p-\sqrt{\frac{D_1}{D_0}}}{p+\sqrt{\frac{D_1}{D_0}}}\right)^n \exp\left\{\frac{-(2n+1)^2h^2}{4D_1t}\right\}$$

$$-\frac{2C_{in}h}{p+\sqrt{\frac{D_1}{D_0}}}\sum_{n=0}^{\infty}\left(\frac{p-\sqrt{\frac{D_1}{D_0}}}{p+\sqrt{\frac{D_1}{D_0}}}\right)^n (2n+1)\cdot\text{erfc}\left\{\frac{(2n+1)h}{2\sqrt{D_1t}}\right\} \qquad \text{Eq. 11}$$

For large $t$, Eq. 11 becomes

$$Q = 2C_{in}\sqrt{\frac{D_0t}{\pi}} - \left(\frac{D_0}{D_1}\right)pC_{in}h \qquad \text{Eq. 12}$$

Therefore the lag time due to drug accumulation in the diffusion layer is given by Eq. 13.

$$\text{Lag time} = \left(\frac{D_0}{D_1}\right)^2\frac{p^2h^2\pi}{4D_0} \qquad \text{Eq. 13}$$

## Discussion

Lag time calculated in this note is a measure of the period required for building-up of drug concentration in the diffusion layer. Another lag time is present when the diffusion constant of the drug in the membrane is smaller than that in the bulk. Appendix of the previous report[6] deals with such a type of lag time.

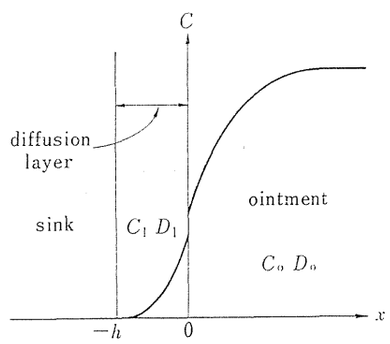


Fig. 1. Concentration Profile Existing in An Ointment, which is in Contact with A Perfect Sink, and in Adjacent Diffusion Layer

5) H.S. Carslaw and J.C. Jaeger, "Conduction of Heat in Solids," Oxford University Press, London, 1959, pp. 319—326.
6) T. Koizumi and W.I. Higuchi, *J. Pharm. Sci.*, 57, 87 (1968).

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# Detoxication Capacity of a Multiple (w/o/w) Emulsion for the Treatment of Drug Overdose: Drug Extraction into the Emulsion in the Gastro-intestinal Tract of Rabbits

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The drug extraction ability of water-in-oil-in-water emulsion was evaluated *in vitro* and *in vivo*. *In vitro* drug extraction into the emulsion was determined using a dialysis system and was significant compared to the control. Blood concentration of salicyclic acid, selected as a model drug, co-administered with the multiple emulsion to rabbits was significantly lower than that in the control. The *in vitro* and *in vivo* experimental results suggest that the emulsion may be useful for the emergency treatment of drug overdose.

**Keywords**——w/o/w emulsion; detoxication; drug overdose; drug extraction; salicylic acid; emergency treatment for drug overdose; rabbit

Poisoning due to drug overdose is a continuing problem. The present modes of emergency treatment are aimed at removal of the drug from the body by various methods, *e. g.* peritoneal dialysis, ingestion of adsorbants such as activated charcoal, and administration of emetics. However, these methods have limitations.

Water-in-oil-in-water (w/o/w) emulsion represents a potential new drug-carrier system with the ability to facilitate gastro-intestinal absorption.[2] Asher *et al.*[3] also showed the utility of such a multiple emulsion for the removal of uremia toxins. Recently, Frankenfeld *et al.*[4] reported the *in vitro* removal of salicylates and barbiturates by w/o/w emulsion, and they suggested that the emulsion was capable of rapid uptake of the drug *in vitro*.

We therefore selected salicylic acid as a model drug and measured the blood levels following oral administration of water-in-oil-in-water emulsion in rabbits to investigate the feasibility of Frankenfeld's suggested emergency treatment for drug overdose *in vivo*.

1) Location: *1-1, Keyakidai, Sakado, Saitama, 350-02 Japan.*
2) R.H. Engel, S.J. Riggi, and M.J. Fahrenbach, *Nature* (London), **219**, 856 (1968).
3) W.J. Asher, K.C. Bovee, J.W. Frankenfeld, R.W. Hamilton, L.W. Henderson, P.G. Holtzapple, and N.N. Li, *Kidney Int.*, 7, s-409 (1975).



## Experimental

**Materials**——Salicylic acid was of J.P. grade, and was used after passage through a No. 150 test sieve. Liquid paraffin was used as an oil phase for the emulsion. A nonionic surfactant, Arlacel C (Tokyo Kasei Kogyo Co.), and a cationic surfactant, cetyltrimethylammonium bromide (CTAB) (Wako Pure Chemical Industries, Ltd.), were selected as the stabilizing agent and dispersing agent for the continuous aqueous phase, respectively.

**Preparation of the Multiple Emulsion**——Twenty % (v/v) Arlacel C in liquid paraffin as an oil phase and 0.1 N NaOH as a central aqueous phase (1 : 1) were mixed and stirred with a magnetic stirrer for 5 min to prepare the w/o emulsion (1st emulsification). Nine volumes of 0.01 N HCl containing 0.05% CTAB (continuous aqueous phase) were added to the resulting emulsion and mechanical agitation completed the formation of the w/o/w dispersion (2nd emulsification) (Chart 1).

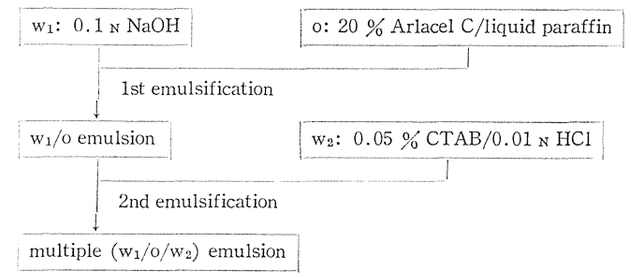


Chart 1. Schematic Diagram of the Preparation of the w/o/w Emulsion

***In Vitro* Experiment**——*In vitro* drug extraction into the emulsion was determined by dynamic dialysis through a cellulose membrane (Visking Co.) (available area = 3.80 cm$^2$). The diffusion cell used in the present experiments is shown in Fig. 1. In cell A, 50 ml of emulsion was mixed with 50 ml of 0.05% CTAB/0.01 N HCl solution at a drug concentration of 100 mg/50 ml or 1000 mg/50 ml, and 100 ml of 0.01 N HCl was placed in cell B. The whole system was maintained at 37±1° using a water bath. At appropriate intervals, aliquots of 1 ml of HCl solution were withdrawn from cell B and determined spectrophotometrically.

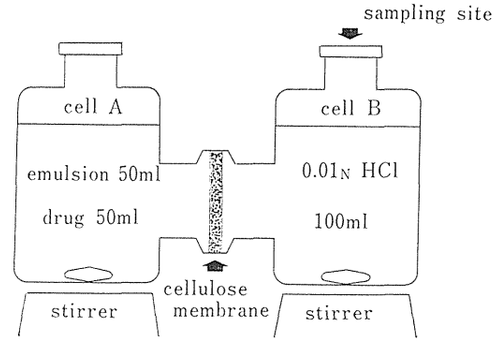


Fig. 1. Apparatus used for the Extraction of Salicylic Acid into w/o/w Emulsion

***In Vivo* Experiment**——Unanesthetized male white rabbits weighing 2 to 3 kg were used. Food was withheld for 24 hr before each experiment. Salicylic acid was suspended in 0.01 N HCl with 0.05% CTAB at a concentration of 20 mg/ml. This suspension was prepared immediately prior to administration and given through a catheter leading into the stomach (16 mg/kg). The emulsion or 0.01 N HCl as a control (9 volumes) was orally administered at the same time. At appropriate times, venous blood samples (0.9 ml each) were collected from an ear vein with a one ml syringe containing 0.1 ml of 3.8% sodium citrate solution and assayed, for drug content.

4) *a*) J.W. Frankenfeld, G.C. Fuller, and C.T. Rhodes, *Drug Dev. Commun.*, **2**, 405 (1976); *b*) C.-W. Chiang, G.C. Fuller, J.W. Frankenfeld, and C.T. Rhodes, *J. Pharm. Sci.*, **67**, 63 (1978).

**Analytical Methods**——Salicylic acid samples obtained during *in vitro* extraction experiments were determined colorimetrically at 530 nm using a Hitachi 100-20 spectrophotometer after reaction with $FeCl_3$ solution. Blood samples were assayed using a Hitachi 650-10S spectrofluorophotometer by the method of Rowland *et al.*[5]

## Results and Discussion

The emulsions prepared as described above are milk-white, and contain spherical oil drops (w/o drops) independently dispersed in the continuous aqueous phase (Fig. 2). The diameter of the oil drops was mostly in the range of 12 to 30 $\mu$m, and the average diameter was 19 $\mu$m.

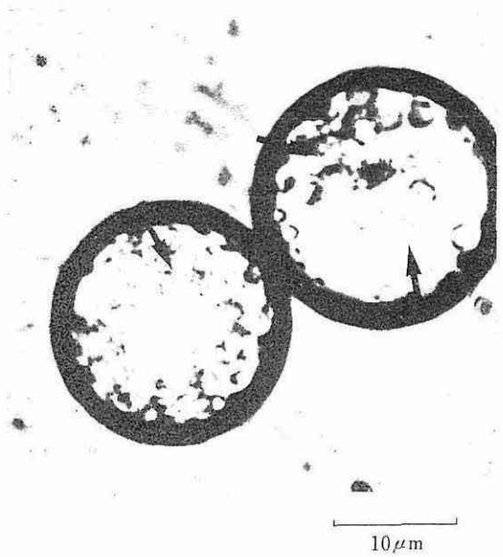


Fig. 2. Photomicrograph of Oil Drops Dispersed in the Continuous Aqueous Phase

Arrows indicate the central aqueous phase.

The emulsion was stable during slow stirring, but if stirring was stopped, it separated into two layers and the oil phase was gradually segregated into the upper layer. Dilute emulsions were more stable than concentrated emulsions. Because salicylic acid is a weak acid, 0.1 N NaOH was selected as a trapping agent for the central aqueous phase ($w_1$), and this agent did not influence the stability of the emulsion.

In the case of *in vitro* drug extraction experiments into emulsions, Fig. 3 shows the amounts of salicylic acid that passed from cell A to cell B through a cellulose membrane with trapping by the emulsion. The amounts of drug in cell B apparently decreased when emulsion was present in cell A. In Fig. 3a (solution type), the initial amount in cell A was 100 mg (per 100 ml) and all of the drug

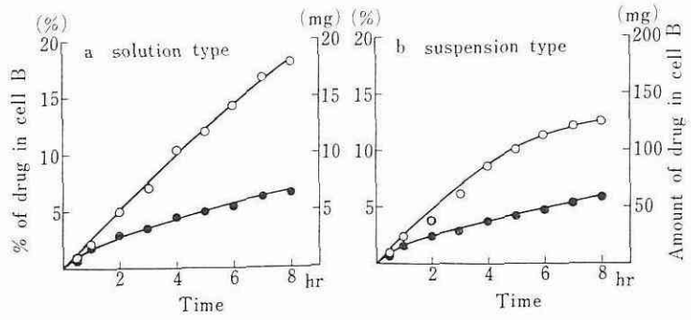


Fig. 3. The Effect of Extraction of Salicylic Acid into the Emulsion on Its Permeability through a Cellulose Membrane

●; with emulsion, ○; control.

5) M. Rowland and S. Riegelman, *J. Pharm. Sci.*, **56**, 717 (1967).



was dissolved in the solution. However, in Fig. 3b (suspension type), the initial amount was 1000 mg; about 22% of the drug was dissolved in solution and 78% was solid particles. These figures confirm the ability of the emulsion to remove salicylic acid.[6]

The time course of blood concentration of salicylic acid following oral administration into rabbits is shown in Fig. 4. The blood concentration in rabbits treated with the emulsions was lower than that in the control. The results suggest that the emulsion may be useful for the emergency treatment of drug overdose. Moreover, the emulsion can be readily prepared using simple equipment, such as an ordinary kichen blender, and thus might be useful as an emergency home treatment for drug overdose. Based on the data obtained in rabbits treated with and without emulsions, we compared the peak concentrations, the times of the peak concentrations, and the areas under the blood concentration-time curves (AUC), as shown in Table I. The peak concentration following co-administration of salicylic acid and emulsion was 40% less than that in the control. The peak concentration is the most important factor in the onset of poisoning. The decrease in the value in rabbits treated with the emulsion is consistent with a process of detoxication. The time at which the peak concentration was established was about 1 hr, and this indicates that the absorption step was completed earlier than in the control, which is consistent with a decrease of absorbable drug content in the stomach due to extraction of the drug into the emulsion. The area under the curve from

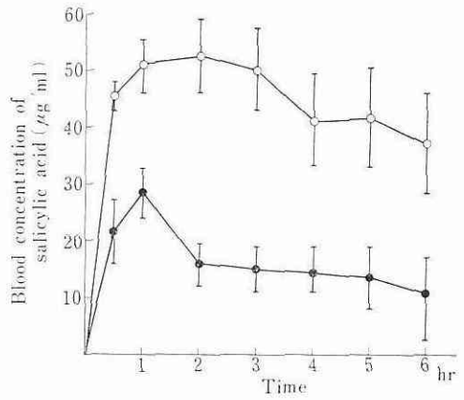


Fig. 4. Time Course of Blood Concentration of Salicylic Acid after Oral Administration

●; with emulsion, ○; control.
Each point represents the mean value of four experiments. Vertical bars indicate ± S. E. M.

Table I. The Peak Concentration, the Time of the Peak Concentration and the Area under the Blood Concentration-time Profiles after Oral Administration of Salicylic Acid

| | | Peak concentration (μg/ml) | Time of peak concentration (hr) | $AUC_{0-6}$ (μg hr/ml) |
|---|---|---|---|---|
| Control | 1 | 56.5 | 2 | 250.9 |
| | 2 | 40.6 | 0.5 | 199.9 |
| | 3 | 64.2 | 1 | 356.6 |
| | 4 | 62.8 | 3 | 354.0 |
| Mean ± S. E. M. | | 56.7 ± 5.4 | | 290.4 ± 38.9 |
| Emulsion | 1 | 30.7 | 1 | 89.6 |
| | 2 | 22.4 | 1 | 116.4 |
| | 3 | 39.6 | 1 | 83.7 |
| | 4 | 29.2 | 2 | 148.3 |
| Mean ± S. E. M. | | 30.5 ± 3.5 | | 109.5 ± 14.8 |
| Significance | | $p < 0.01$ | | $p < 0.01$ |

6) The dissolution of solid drug particles, drug extraction into the emulsion, and dialysis through a Visking membrane all obey first-oder kinetics. An analysis of the data as shown in Fig. 3 could be simulated using the compartment model with an analog computer, *etc.* Thus, the time course of extraction of the drug into the emulsions should be predictable. These results will be reported elsewhere.

0 to 6 hours was measured by the trapezoidal method. The resulting $AUC_{0-6}$ was 46% less than that in the control experiments. The values of the peak concentration and $AUC_{0-6}$ were significantly different from those of the control ($p<0.01$).

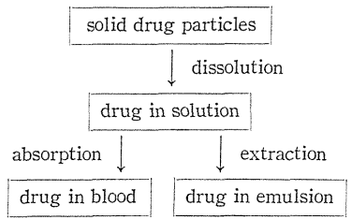


Chart 2. The Processes of Absorption and Extraction into the Emulsion after Ingestion of Salicylic Acid

Upon administration of excess drug, however, a large portion remains as a solid in the stomach, so the dissolution rate of the drug might affect the drug extraction capability of the emulsion (Chart 2). If the extraction potential into the emulsions is saturated, the unextracted portion of the drug will be absorbed into the blood. This might be overcome by continuous infusion of the emulsion.

The amount of drug absorbed into the blood and extracted into the emulsions in the stomach were influenced by the gastric contents. In our experiments, rabbits were fasted during the entire day before oral administration. However, there was some gastric content due to coprophagy by the rabbits, and this might affect the stability of the emulsion in the stomach with resulting changes in the blood level of the drug.

Chiang *et al.*[4b] reported *in vitro* studies showing that the extraction of a drug into the emulsion decreased in the presence of bile salt. We did not observe any effect of bile salts on drug extraction by the emulsion *in vivo* in this study, but further work is planned.

These experimental results suggest that w/o/w emulsions may be useful for the emergency treatment of drug overdose. However, there are still many difficulties facing clinical trials of the emulsion. Studies are presently under way in our laboratory to improve the stability of the w/o/w emulsion and to make it capable of rapid uptake of many weakly acidic drugs. In addition, we plan to investigate the usefulness of the emulsion following ingestion of basic drugs or co-administration of two or three drugs *in vivo*.

# Disopyramide phosphate (DIP-P) の体内動態（第Ⅰ報）
## ラットにおける吸収，分布及び排泄

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（昭和55年11月29日受理）

# Studies on the Metabolic Fate of Disopyramide phosphate (DIP-P) I
## Absorption, Distribution and Excretion in Rats

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Yuko NAKAMURA*, Kunio NAKANISHI,
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and Motonobu NAKAJIMA***

**Summary**

The absorption, distribution and excretion of an antiarrhythmic agent, disopyramide phosphate (4-diisopropylamino-2-phenyl-2-(2-pyridyl)-butylamide phosphate, DIP-P) in male rats were investigated using the $^{14}$C-labeled compound. The elimination process of radioactivity in whole blood was divided into three phases after intravenous administration of DIP-P. The absorption of DIP-P after oral administration was slower than that of free base. Cumulative biliary excretion was 23.0% within 0.5 hr and 61.8% within 8 hr after intravenous administration of DIP-P. Urinary and fecal excretion of radioactivity during 48 hr after intravenous administration of DIP-P was 34.7% and 61.2% of the given dose, respectively. And excretion profiles after oral administration of DIP as a free and phosphate form exhibited the same curve. Tissue distribution of radioactivity in the small intestine and stomach after the injection of DIP-P was higher than that in other tissues.

**Key words**

Disopyramide phosphate, 4-diisopropylamino-2-phenyl-2-(2-pyridyl)-butylamide phosphate, Intravenous or oral administration, Blood level, Tissue distribution, Urinary or biliary excretion, Rats

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## 緒　言

不整脈治療剤として開発された disopyramide (4-diisopropylamino-2-phenyl-2-(2-pyridyl)-butylamide, DIP-F) は，1951年 H. W. Sause により合成された化合物であり，1962年 Mokler らによってある種の心律動障害の治療に有効な新しい抗不整脈剤として報告された[1]．この薬物の吸収，分布，排泄並びに代謝については，Ward ら (1977)[2] や立松ら (1975)[3] によって検討が試みられているものの，薬物動力学的観点からの検討を含めた詳細な実験はなされていない．最近この disopyramide phosphate (DIP-P) が開発され，その臨床応用が待たれているが，DIP-P についての経口及び静脈内投与後の吸収，分布，排泄の詳細な知見が必要とされている．そこで著者らは，$^{14}$C-disopyramide phosphate ($^{14}$C-DIP-P) を用いて，ラットに経口投与あるいは静脈内投与した際の血中濃度，体内分布及び排泄について検討した．なお比較のため $^{14}$C-disopyramide-free ($^{14}$C-DIP-F) の経口投与も行った．

## 実験方法

### 1．標識化合物

$^{14}$C-DIP-P 及び $^{14}$C-DIP-F は，Roussel-Uclaf 社より提供されたもので，その構造式及び標識位置を Fig. 1 に示した．両化合物の比放射能は，58.3 mCi/mol であった．また，クロロホルム/メタノール/酢酸（40：10：2）を展開溶媒とし，シリカゲルを用いた薄層クロマトグラフィー法によって検定を行った結果，$^{14}$C-DIP-P 及び $^{14}$C-DIP-F の放射化学的純度は各々97.7%及び98.1%であった．

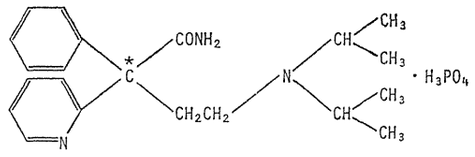


**Fig. 1** Chemical Structure of Disopyramide Phosphate and the Position of Radioactive Labeling (*)

### 2．実験動物及び投与法

実験動物は体重190～210g のウィスター系雄性ラットを用いた．動物は恒温（22±1°C）及び恒湿（55±5%）の飼育室で自由に餌と水を与え，約1週間予備飼育した後，実験に供した．

投与薬物は，$^{14}$C-DIP-P に非放射性 DIP-P を加え，また $^{14}$C-DIP-F に非放射性 DIP-F を加えて生理食塩水に溶解して2.28μM/m*l*（DIP-P：1.0mg/m*l*, DIP-F：0.774mg/m*l*）とし，放射活性を20μCi/m*l* になるように調製した．経口投与の場合は，この試料溶液を1.0m*l*/kg として一夜絶食させたラットに経口ゾンデを用いて強制投与した．また静脈内投与の場合は，エーテル麻酔下で頸静脈を露出後，1m*l*/kg を注入した．胆管を結紮したラット及び胆管にポリエチレン管（PE10, Clay Adams 社）を挿入したラットにおける静脈内投与は，ソモノペンチル0.5m*l*/kg をラット腹腔内投与によって麻酔した後，頸静脈から先に調製した試料溶液を1.0m*l*/kg 注入した．なおラットは1群5例以上とした．

### 3．血中濃度の測定

採血は，経時的に経口投与の時は頸静脈から，また静脈内投与の時は尾静脈から行った．採血量は経口投与時 0.5m*l*，静脈内投与時 20μ*l* とした．血液に Protosol®・エタノール混液（1：2の容積比）2m*l* を加え（静脈内投与時0.25m*l*），55°Cで1時間インキュベートし，室温にもどし30%過酸化水素水 1m*l*（静脈内投与時0.1m*l*），再度55°C で30分インキュベートする．次いで Aquasol®-2 15m*l*（静脈内投与時 10m*l*）を加えて激しく撹拌し，更に 0.5N-塩酸1.5m*l* を加えて一夜放置した後，放射活性を測定した．

### 4．尿及び胆汁中濃度の測定

ラットの尿は，ボールマンゲージ（夏目，Ⅲ型）に固定した後，所定の時間に採取した．胆汁は，ソモノペンチル麻酔下，背位固定して胆管にカニューレを挿入し，所定時間に採取した．

尿及び胆汁は，水で定容積に希釈し，その1m*l* をバイアル管にとり，Aquasol®-2 10m*l* を添加し，激しく撹拌した後，一夜放置し，その放射活性を測定した．

### 5．糞中濃度の測定

ラットをボールマンゲージに固定し，所定時間ごとに糞を集めた．それらの重量を測定した後，0.5N-塩酸 50m*l* を加えて室温で1時間撹拌し，静置後その上

清0.5m*l* と Aquasol®-2 10m*l* を加えて激しく撹拌した後，更に一夜放置しその放射活性を測定した．

### 6．組織中濃度の測定

所定時間に断首放血死させ，各組織を摘出し，湿重量を測定した後，0.1g をとり，バイアル管に入れて Protosol® 1m*l* を添加し，55℃ で2時間インキュベートする．室温にもどした後，30% 過酸化水素水 0.1 m*l* を加えて再度55℃ で30分インキュベートする．次いで Aquasol®-2 10m*l* を加えて激しく撹拌し，一夜放置後その放射活性を測定した．

### 7．放射活性の測定

液体シンチレーションカウンター(Aloka LSC-651)で放射活性の測定を行った．測定効率の補正は，外部標準線源比法により行った．得られた放射活性値は，投与量に対する割合として示した．

## 実験成績

### 1．血中濃度―時間曲線

ラットに DIP-P を静脈内投与し，経時的に測定した血中濃度の推移を Fig. 2 に示した．血中放射活性濃度は投与後急速に，その後はゆるやかに減衰していったが，12時間目非常にゆるやかな極大が現われた．この極大を無視した場合，差引残余法により，血中濃度―時間曲線の式は，

$$C = 0.055\ \exp(-5.279t) + 0.025\ \exp(-0.667t) + 0.014\ \exp(-0.014t)$$

と求められ，3相性に減衰することが判った．これにより，血中からの消失半減期は各々の相で0.13，1.04，及び49.50時間と計算された．

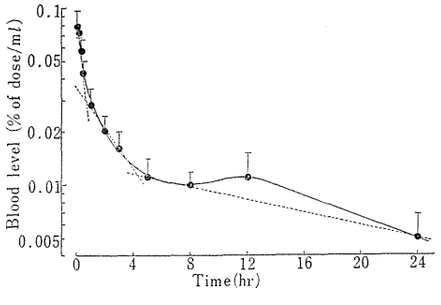


**Fig. 2**　Semilogarithmic Plots of Blood Level of Radioactivity after Intravenous Injection of $^{14}$C-Disopyramide Phosphate in Rats

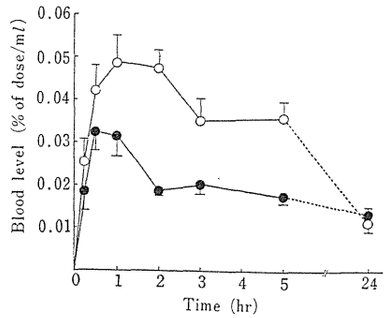


**Fig. 3**　Blood Level of Radioactivity after Oral Administration of $^{14}$C-Disopyramide Phosphate and Free Base in Rats
Each point represents the mean ± S. E. of 7 to 10 rats
—●—; phosphate, —○—; free base

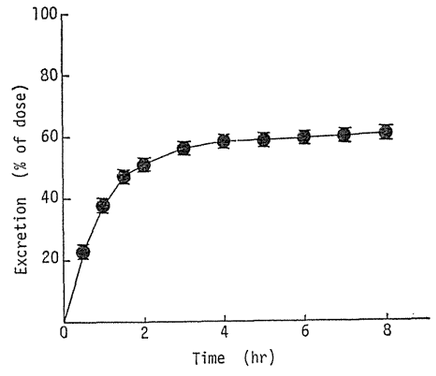


**Fig. 4**　Cumulative Biliary Excretion of Radioactivity after Intravenous Injection of $^{14}$C-Disopyramide Phosphate in Rats
Each point represents the mean ± S. E. of 5 rats

一方，DIP-P 及び DIP-F を溶液状態で経口投与したラットの血中放射活性濃度の時間推移を Fig. 3 に示した．両薬物ともに消化管ですばやく吸収されて，最高血中濃度到達時間は DIP-P で投与後30分，DIP-F では1時間であったが，ピーク以降の血中からの消失は比較的ゆるやかであり，先の DIP-P 静脈内投与後の消失パターンとは異なっていた．また，投与後24時間までの血中濃度は，DIP-F の方が DIP-P よりも高かった．DIP-P は DIP-F に比べ，溶解度がはるかに大きいという利点をもつが，本実験では両薬物共水溶





液状態で投与したので，消化管内における溶解性が，血中濃度に影響したとは考えにくい．血中濃度に差が生じた原因の1つとして，DIP-P の方の脂溶性の低さが予期されるが，この因子の吸収への寄与については現在明らかでない．

### 2．胆汁中排泄

DIP-P の静脈内投与後の胆汁中累積排泄量の平均値を Fig. 4 に示した．累積排泄量は，投与後30分で23.0%，3時間で56.1%，8時間では61.8%であった．

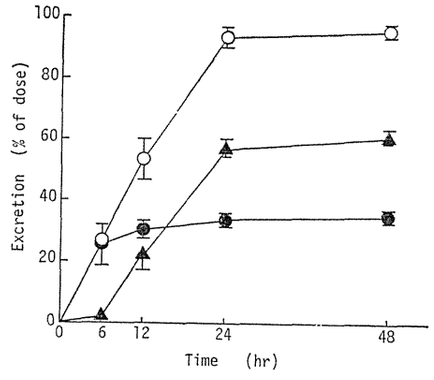


**Fig. 5**　Urinary and Fecal Excretion of Radioactivity after Intravenous Injection of $^{14}$C-Disopyramide Phosphate in Rats
Curves (—●—; urine, —▲—; feces, —○—; total) are cumulative amount of radioactivity
Each point represents the mean ± S. E. of 5 rats

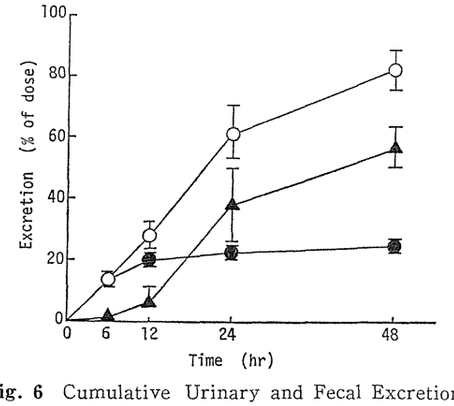


**Fig. 6**　Cumulative Urinary and Fecal Excretion of Radioactivity after Oral Administration of $^{14}$C-Disopyramide Phosphate in Rats
Each point represents the mean ± S. E. of 6 rats　Key: as in Fig. 5

### 3．尿及び糞中排泄

DIP-P を静脈内投与した際の，尿及び糞への累積

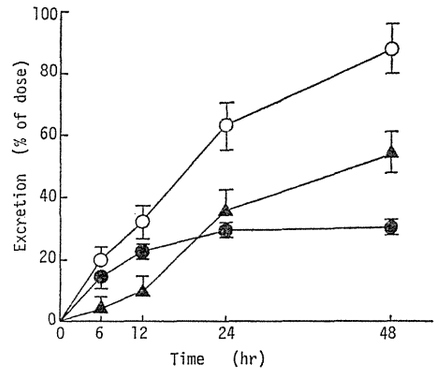


**Fig. 7**　Urinary and Excretion of Radioactivity after Oral Administration of $^{14}$C-Disopyramide Free Base in Rats
Each point represents the mean ± S. E. of 6 rats　Key: as in Fig. 5

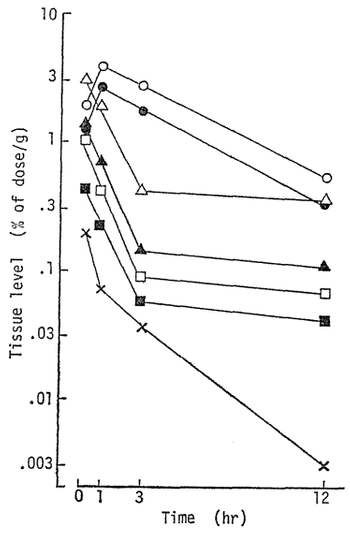


**Fig. 8**　Semilogarithmic Plots of Tissue Distribution of Radioactivity after Intravenous Injection of $^{14}$C-Disopyramide Phosphate
—○—; intestine, —●—; stomach, —△—; liver, —▲—; kidney, —□—; lung, —■—; heart, —×—; brain

排泄量の平均値を Fig. 5 に示した．2日間で投与放射活性の95.9％が排泄され，尿中は34.7％，糞中に61.2％であり，糞中への排泄の多いことが明らかとなった．

また，DIP-P 及び DIP-F の経口投与後の尿及び糞への累積排泄量の平均値を Fig. 6 及び Fig. 7 に示した．投与後48時間までに DIP-P では総累積排泄量が83.1％（尿中へ25.5％，糞中へ57.6％）であり，DIP-F の総累積排泄量は，87.6％（尿中へ30.2％，糞中へ57.4％）となり，いずれの場合も尿中より糞中への排泄量の方が多かった．

一方，胆管を結紮した後，DIP-P を静脈内投与したラットにおける排泄は，投与後48時間までに尿中累積排泄量の平均値が94.2％となり，糞中累積排泄量の平均値は4.6％となって，糞中排泄が著しく低下し，糞中排泄は胆汁排泄の結果生じることが推測された．

### 4．組織中濃度

DIP-P の静脈内投与後の組織内放射活性濃度の推移を Fig. 8 に示した．組織への移行は速やかであった．投与直後，放射活性は，肝臓が一番高く，以下小腸，腎臓，胃，肺，心臓，脳の順に低下していた．しかし，肝臓，腎臓，肺及び心臓では，3時間までその放射活性がすみやかに減衰し，それ以後12時間までゆるやかに減衰するという2相性を示すのに対して，胃及び小腸では，投与後1時間で最高濃度を示した後，12時間までゆるやかに減衰していった．脳は，測定した組織のうちで一番濃度が低かった．また，図に示した組織中濃度は，脳の12時間の値を除くと，いずれも血液中濃度より高かった．

## 考　察

DIP-P を雄ラットに静脈内投与した場合，放射活性は，血中から急速に消失したが，12時間目非常にゆるやかな極大が認められた．このゆるやかな極大にとらわれずに差引残余法で．血中濃度―時間曲線の式を求めると，放射活性の消失過程は3相に分離することができた．すなわち，第1相では血中半減期が0.13時間，第2相1.04時間，第3相49.5時間と計算された．

また，12時間目にゆるやかな極大が生じた原因の1つとしては，Fig. 4 に示したように胆汁中排泄が比較的すみやかであることと，Fig. 8 に示した組織内分布の実験において，小腸では静脈内投与後1時間で極大値をもつということより，腸肝循環の存在が推測される．

一方，DIP-P 及び DIP-F を溶液状態で経口投与した場合の血中濃度―時間曲線は，静脈内投与の場合とは異なり，投与直後から30分にかけての血中濃度の急激な立ち上りとその後のゆるやかな減少というパターンを示した．血中濃度の急激な立ち上りが存在することは，DIP-P 及び DIP-F の消化管吸収（塩基性薬物であることを考えると，小腸が主要吸収部位と推測される）が，比較的すみやかに起こることを意味している．また，その後の血中濃度がゆるやかに減少することは，この薬物が胆汁排泄をかなり受けることより（Fig. 4）腸肝循環によることも予想されるが，静脈内投与の場合の腸肝循環の可能性が定かでないため，この寄与については現在明らかでない．他の推測としては，この薬物が，弱い抗コリン作用[1,4]を有することより，胃から小腸への薬物溶液の移行が遅れたために，小腸での吸収がゆっくりと持続して行われたということも考えられる．

DIP-P の静脈内投与後の胆汁中累積排泄量は比較的多く，投与後30分で23.0％，3時間で56.1％，8時間では61.8％となった．Karim[5]らは，DIP-P代謝物の検索を行い，ラット胆汁中では代謝物が大部分を占め，未変化体が少ないことを報告している．Karim の報告と今回の実験結果を合せ考えると，血液中から肝組織に取り込まれた薬物は，大部分が比較的すみやかに代謝されて胆汁中へ排泄されるため，Fig. 5 に示したような8時間で61.8％という盛んな胆汁排泄が起こるのではないかと推測される．

一方，尿及び糞中累積排泄量をみると，DIP-P 静脈内投与後48時間までに尿中へ34.7％，糞中へ61.2％と，糞中へ排泄された量がはるかに多かった．このような尿より糞への排泄量が多いのは，DIP-P 及び DIP-F の経口投与後の尿糞中排泄量においても同じ傾向であったが，これは血液から胆汁中への排泄が比較的速く，またその量が多い（Fig. 4）ことに起因していると考えられる．このことは，胆管結紮をほどこしたラットに DIP-P を静脈内投与すると，投与後48時間に糞中排泄量がわずか4.6％であることからも支持される．また，DIP-P の静脈内投与後8時間までの胆汁中累積排泄量61.8％と48時間までの糞中累積排泄量61.2％の値が極めて近似していることは興味あることである．なお，尿中及び糞中を合わせると DIP-P の静脈内投与後24時間で92.2％，48時間で95.9％が排泄され DIP-P の蓄積性は少ないことが示された．





DIP-P の静脈内投与後の組織内分布は，血液中から組織への移行がすみやかであるパターンを示した．肝臓，腎臓，肺，心臓及び脳は，15分ですでに高い放射活性を示し，その後12時間まで直線的に減衰していった（Fig. 8）．このことから，DIP-P は，小腸及び胃とその他の組織とでは親和性の異なることが判明した．また，脳内放射活性は，血液中を除くと測定した他の組織のどれよりも低く，投与後12時間で痕跡程度であった．

## 結　論

DIP-P をラットに静脈内投与して，また DIP-P 及び DIP-F を経口投与して吸収，分布，排泄について検討し，以下の結果を得た．

1．静脈内投与後，放射活性はすみやかに組織に移行し，血中濃度は12時間目の極大を無視すると3相性の減少を示した．

2．経口投与後，DIP-P 及び DIP-F はすみやかに吸収されて，30分から1時間で最高血中濃度に達するが，その後の減少は比較的ゆるやかであることが判明した．

3．静脈内投与後の胆汁中排泄は，すみやかに起こり，投与後8時間で61.8％が排泄された．

4．静脈内投与後の尿及び糞中排泄の48時間での総和は95.9％であり，糞中の方が尿中よりも多かった．この傾向は，DIP-P 及び DIP-F の経口投与後の尿及び糞中排泄においても認められたが，累積排泄量の値から蓄積性の少ない薬物であることが明らかとなった．

5．静脈内投与後の組織内分布は，投与後15分で高い放射活性を示す肝臓，腎臓，肺，心臓及び脳と1時間で高い放射活性を示す小腸と胃の2つに分けることが可能であり，組織親和性に差のあることが明らかとなった．

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# Disopyramide phosphate (DIP-P)の体内動態（第Ⅱ報）*
## ラットにおける静脈内投与後の血中濃度，全身オートラジオグラフィー及び乳汁移行

森本　雍憲，杉林　堅次，中村　祐子**，中西邦夫
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4

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（昭和55年11月29日受理）

4

# Studies on the Metabolic Fate of Disopyramide phosphate (DIP-P) II*
## Blood Concentration, Whole Body Autoradiography and Transfer via Milk after Single Intravenous Administration of $^{14}$C-DIP-P in Female and Pregnant Rats

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**Summary**

The absorption, distribution and excretion of disopyramide phosphate (DIP-P) were investigated in pregnant rats after intravenous administration of $^{14}$C-DIP-P. The elimination process of radioactivity in whole blood was devided into two phases. The excretion of radioactivity was about 47.0% in feces and about 37.5% in urine 48 hr after the administration. When $^{14}$C-DIP-P was administered intravenously to pregnant and lactating rats, radioactivity was found in the fetus of pregnant rats and in the milk of lactating rats.

Whole body autoradiogram of pregnant rats revealed that the radioactivity was transferred to the fetus through the placenta.

**Key words**

Disopyramide phosphate, Absorption, Distribution, Excretion, Pregnant rats

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## 緒　言

4-diisopropylamino-2-phenyl-2-(2-pyridyl)-butylamide phosphate (disopyramide phosphate, DIP-P) のラットにおける静脈内投与後の吸収，分布及び排泄について，DIP-P 及び disopyramide の経口投与後の吸収並びに排泄の挙動も合せて第1報[1]において報告した．

今回我々は，DIP-Pの妊娠ラットにおける静脈内投与後の血中濃度，尿及び糞中排泄，胎盤通過及び乳汁移行について，全身オートラジオグラフィーも合わせて実験を行い，DIP-Pの生体内動態を検討したので報告する．





## 実験方法

### 1. 標識化合物
第Ⅰ報[1]に記載されたものと同一物を使用した．

### 2. 実験動物
妊娠18日目の Wistar 系ラット（体重320±20g）を用いた．また，乳汁中薬物濃度を検討した実験においては，同系の分娩後9日目のラットの乳汁を用いた．

他は第Ⅰ報[1]に準じて Wistar 系雄性ラットを用いた．これらの動物はいずれも恒温（22±1°C）及び恒湿（55±5%）の環境下で飼育した．

### 3. 投与法
試料溶液は，第Ⅰ報[1]に記載されたように調製したものをそのまま使用した．投与量及び投与法についても同様である．

また，静脈内投与の場合はいずれも非絶食としたが，経口投与の場合は絶食（約16時間）とした．

### 4. 血中濃度の測定
採血は，経口投与並びに静脈内投与の両方において頸静脈から行った．採血量は，妊娠ラット並びに乳汁を採取したラットの両者共に20$\mu l$とした．測定は，前報[1]に記載した操作に準じて行った．

### 5. 糞尿中濃度の測定
ラットの尿及び糞は，ボールマンゲージ（夏目，Ⅲ型）に固定した後，所定の時間に分別採集した．糞尿の処理及び放射活性の測定は，第Ⅰ報[1]に準じて行った．

### 6. 胎仔及び羊水中濃度の測定
妊娠ラットに$^{14}$C-DIP-Pを静脈内投与した後，所定の時間に頸動脈を切断し，直ちに開腹して羊膜に包まれた胎仔を摘出した．また同時に羊水も採取した．胎仔は水で20%ホモジネートを作り，その0.5m$l$を用いてその放射活性を測定した．

### 7. 乳汁中濃度の測定
分娩後9日目のラットを乳仔から離し，試料溶液投与後各々の所定の時間の10分前，ソモノペンチルと oxytocin（Atonin-O®，帝国臓器）の各0.1m$l$を腹腔内に注入した．乳頭を10分間マッサージすることにより乳汁を採取したのち，直ちに後大静脈より採血し，それぞれの放射活性を測定した．

### 8. 全身オートラジオグラフィー
ラットに$^{14}$C-DIP-Pを投与し，所定時間にエーテル麻酔死させ，直ちにドライアイス—アセトンの冷媒中で全身を凍結させると共に，7%カルボキシメチルセルロースペーストに包埋凍結した．これら凍結ブロックから，クライオミクロトームを用いて厚さ40$\mu$の切片を得，凍結乾燥後，工業用X線フィルム（Sakura, Type N）にミラー膜を用いて密着させ，約1カ月間露出して全身オートラジオグラムを得た．

### 9. 放射活性の測定
第Ⅰ報[1]に準じて行った．

## 実験成績

### 1. 血中濃度—時間曲線
妊娠ラットにDIP-Pを静脈内投与し，経時的に測定した血中濃度の推移を Fig. 1 に示した．血中放射活性濃度は投与後急速に，その後はゆるやかに減衰していったが，12時間目に明らかな極大が現われた．前報[1]における雄性ラットへの DIP-P の静脈内投与時の血中濃度においても12時間目でゆるやかな極大が認められたことから，DIP-Pは静脈内投与後12時間目近傍の血中濃度が，その前後より幾分高くなる挙動を示すことが明らかである．この極大を無視した場合，差引

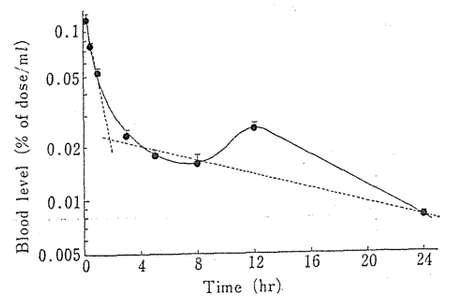


**Fig. 1** Blood level of radioactivity after intravenous administration of $^{14}$C-DIP-P to pregnant rats
Each point represents the mean ± S.E. of 5 to 15 rats

残余法により血中濃度—時間曲線の式は，

$C = 0.104\exp(-1.155t) + 0.022\exp(-0.042t)$

と求められ，2相性に減衰することが判った．これにより，血中からの消失半減期は各々の相で0.60及び16.6時間と計算された．

### 2．糞及び尿中排泄

DIP-Pを妊娠ラットに静脈内投与したときの糞及び尿への累積排泄量の平均値を Fig. 2 に示した．投与後48時間で投与放射活性の80.5%が排泄され，糞中は47.0%，尿中は33.5%となっていて，糞中排泄の方が多いことが明らかとなった．この結果は，雄性ラットへの DIP-P の静脈内投与後の同様の実験結果と類似していた．

### 3．胎仔への移行

静脈内投与後の胎仔及び羊水中放射活性の時間的推移を Fig. 3 に示した．胎仔中の放射活性は，母体血液放射活性が投与直後最大で，以後急速に減衰していくのとほぼ同じパターンで減衰した．羊水中放射活性は胎仔中より低いが，その減衰挙動は，血液中及び胎仔中のそれとほぼ同様であった．

### 4．乳汁への移行

母ラットにDIP-Pを静脈内投与した後，乳汁並びに母ラット血液中放射活性の時間推移を Fig. 4 に示した．乳汁中放射活性は，投与直後高い値を示すが，その減衰は著しく速く，投与後5時間で15分値のおよそ10分の1となった．

この時の血液中放射活性の値と8時間までの減衰挙動は，妊娠ラットのそれとほぼ等しかったが，乳汁中と血液中を比較すると，乳汁中放射活性の方がはるかに高いことが明らかとなった．

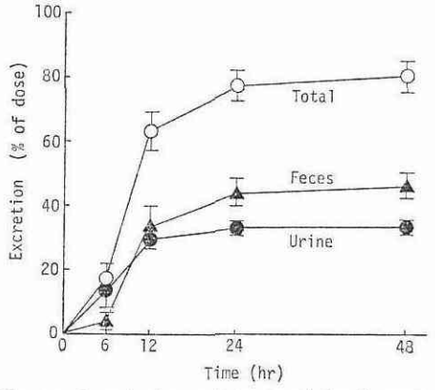


**Fig. 2** Cumulative urinary and fecal excretion of radioactivity after intravenous administration of $^{14}$C-DIP-P to pregnant rats
Each point represents the mean ± S. E. of 6 rats

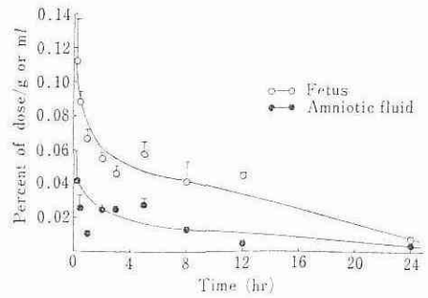


**Fig. 3** Radioactivity in fetus and amniotic fluid after intravenous administration of $^{14}$C-DIP-P to pregnant rats
Each point represents the mean ± S. E. of 4 to 9 rats

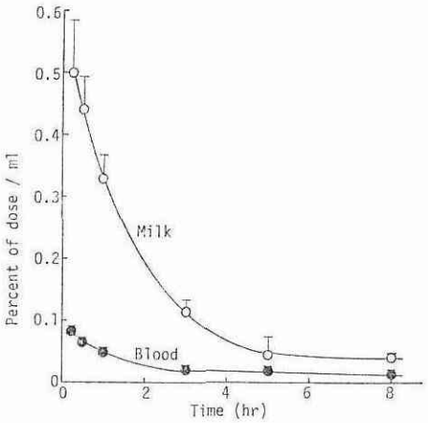


**Fig. 4** Blood and milk levels of radioactivity after intravenous administration of $^{14}$C-DIP-P to lactating rats
Each point represents the mean ± S. E. of 8 rats

### 5．全身オートラジオグラフィー

妊娠18日目のラットに$^{14}$C-DIP-Pを静脈内投与したときの経時的全身オートラジオグラムを Fig. 5 に示した．





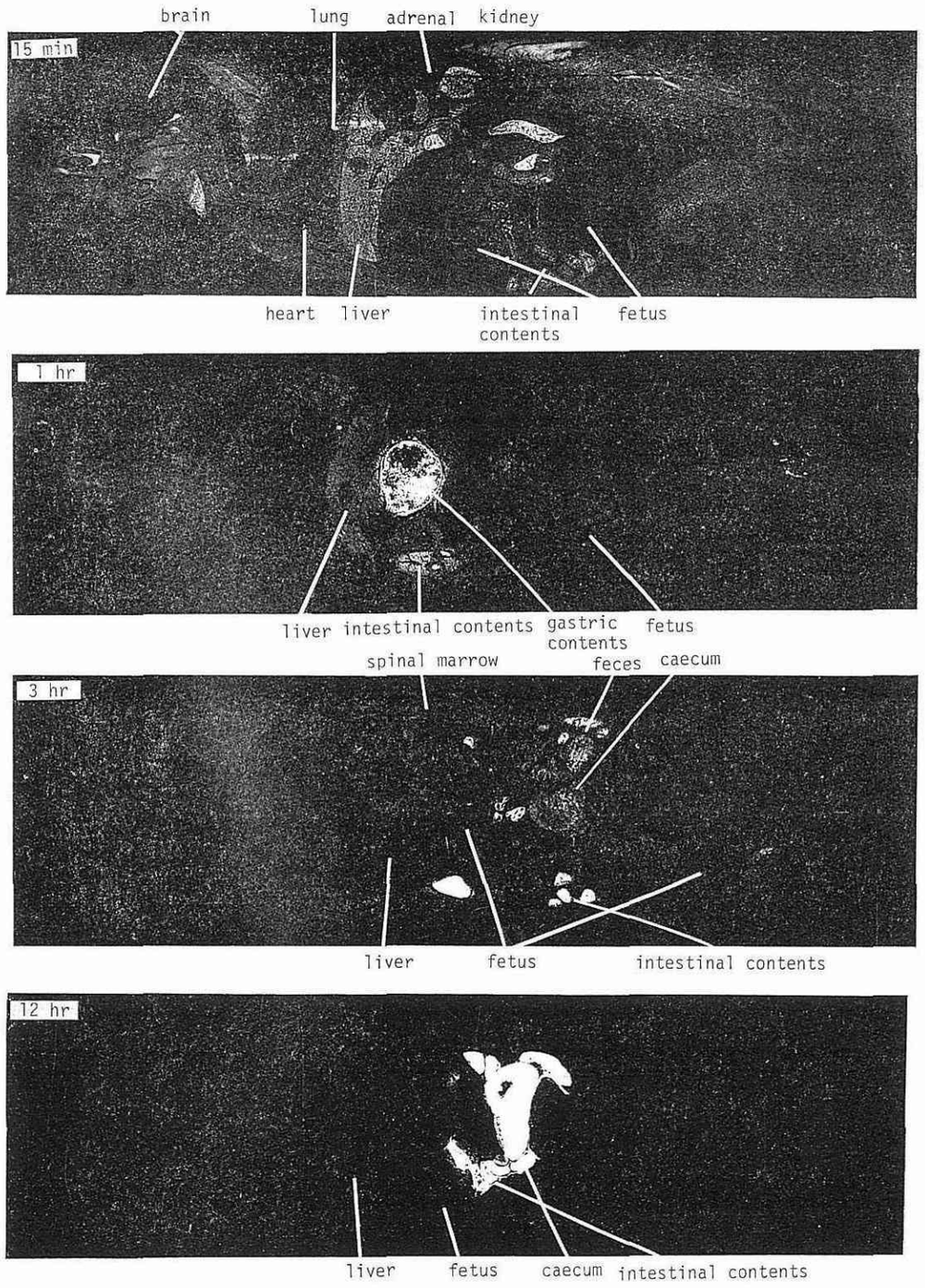


**Fig. 5** Whole body autoradiogram after intravenous administration of $^{14}$C-DIP-P to pregnant rats

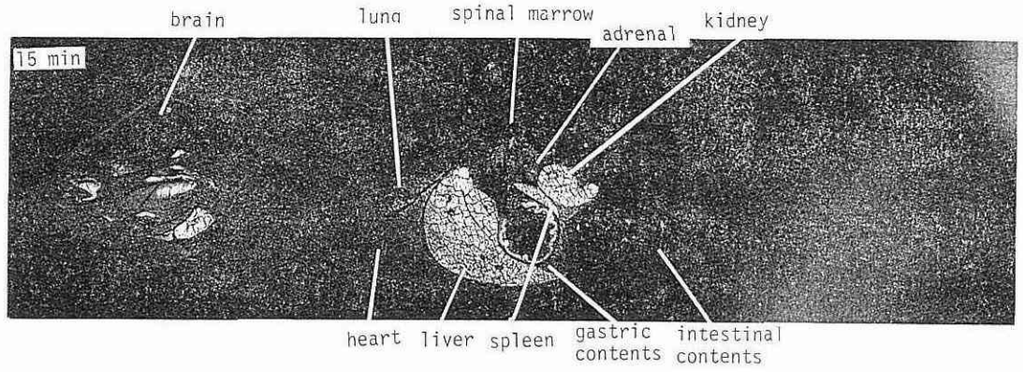


**Fig. 6** Whole body autoradiogram after intravenous administration of $^{14}$C-DIP-P to male rats





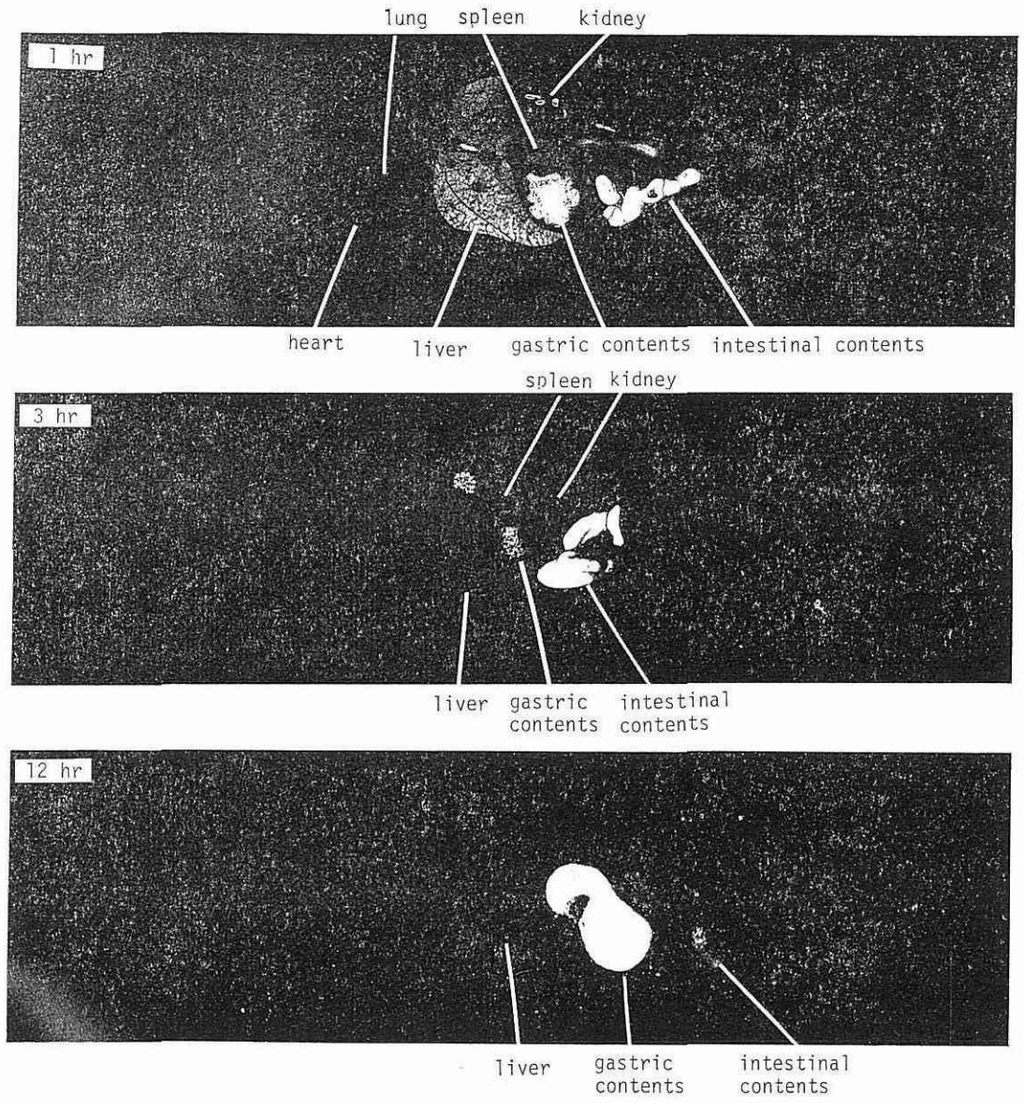


**Fig. 7** Whole body autoradiogram after oral administration of $^{14}$C-DIP-P to male rats

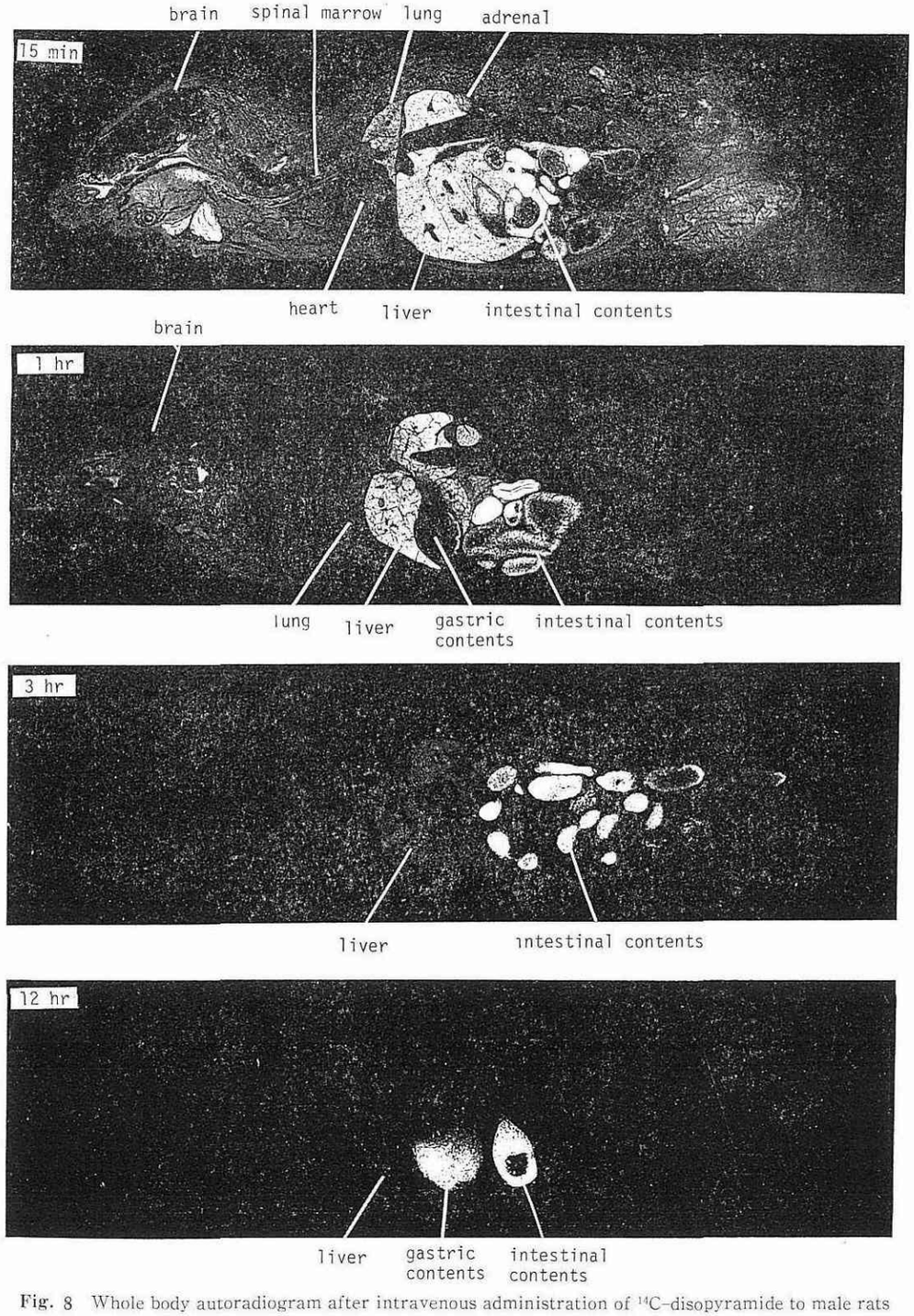


**Fig. 8** Whole body autoradiogram after intravenous administration of $^{14}$C-disopyramide to male rats





した．また，雄性ラットに$^{14}$C-DIP-Pを静脈内投与した時の代表的なものをFig. 6に，$^{14}$C-DIP-Pを経口投与した時のものをFig. 7に，更に，$^{14}$C-disopyramideを静脈内投与した時のオートラジオグラムをFig. 8に示した．

妊娠ラットの場合，投与後15分でほぼ全身に分布し，肝臓，腎臓に比較的高度に分布していたが，胎仔では少なかった．投与後1時間では，胃内容物に高い放射活性が認められ，次いで小腸内容物にも認められた．投与後3時間，12時間と経過するにつれて，胃内容物に認められた高い放射活性は小腸，盲腸へと順次移行し，更には糞にも放射活性が比較的高度に認められたが，その他の臓器及び組織中にはほとんど認められない程度に減少していた．この時間の経過につれて，放射活性が胃から順に消化管内を直腸へ向けて移動するのは，胃内容物の移動と胆汁排泄（この薬物は胆汁中排泄が比較的多い[12]）によるものと推測される．

雄性ラットに$^{14}$C-DIP-Pを静脈内投与した時は，投与後15分で肝臓，腎臓，脾臓，胃に高い放射活性が認められ，小腸及び心臓は低かった．1時間では小腸に高く，肝臓は15分よりも低下した．3時間後では，小腸及び胃に高く，肝臓に中程度認められ，12時間後では小腸に高い放射活性を認めるのみで，他の臓器及び組織中には痕跡程度しか存在しなかった．

$^{14}$C-DIP-P の経口投与後15分では，胃及び小腸に高く，次いで腎臓，肝臓，肺，心臓にも放射活性が認められた．3時間後では，胃から小腸への移行につれて小腸に高い放射活性が存在し，胃にも認められたが，肝臓，脾臓，腎臓はそれ程高くはなかった．12時間後では，胃に比較的高い放射活性が存在し，小腸内容物中にもわずかではあるが活性が認められた．

$^{14}$C-disopyramide の静脈内投与後のオートラジオグラムは$^{14}$C-DIP-Pの静脈内投与の結果と類似しており，投与後15分では，肝臓，肺，小腸に高い放射活性が認められ，次いで副腎，心臓更には脳にもかすかに認められた．1時間では，肝臓，小腸及び胃に高く，他の臓器ではかなり低下し，3時間では，小腸に顕著な放射活性が認められた．12時間後では，小腸と胃に明らかな放射活性が認められた．

## 考　察

DIP-Pを妊娠ラットに静脈内投与した時，放射活性は血中から急速に消失したが，12時間目に明らかな極大が認められた．前報[1]のDIP-Pの雄性ラットへの静脈内投与時の血中濃度においても，12時間目にわずかではあるが極大が認められたことより，disopyramideには血中濃度を増加させる要因，すなわちこの場合胆汁排泄が多いこと[12]から，腸肝循環の存在が推測される．この12時間値にとらわれずに差引残余法で血中濃度—時間曲線の式を求めると，放射活性の消失過程は2相に分離することができた．すなわち第1相では血中半減期が0.60時間であり，第2相では16.6時間であった．雄性ラットの場合は消失過程が3相であったが，妊娠時では多少異なるものと思われる．

一方，糞尿中排泄量をみると，投与後48時間までに尿中へ33.5%，糞中へ47.0%と糞中へ排泄された量がはるかに多かった．この傾向は，前報の雄性ラットへのDIP-Pの静脈内投与時の時とよく類似している．また蓄積性も比較的低いものと思われる．

妊娠ラットにDIP-Pを静脈内投与後15分で放射活性は胎盤を通過し，胎仔に移行することが認められたが，以後胎仔内濃度は，母体血中濃度の急激な低下に比例するようにすみやかに消失していくことが明らかとなった．

更に分娩後9日目の母ラットの静脈内にDIP-Pを投与した時の15分後の乳汁中濃度は，母体血中濃度より約5倍高かったが，その消失は非常にすみやかで15分値に比較すると，5時間値ではおよそ10分の1となっていた．

オートラジオグラムで妊娠ラットへの投与後15分，1時間，3時間，12時間といずれの場合も胎仔への移行は少ないことが明らかとなった．肝臓，腎臓，胃，小腸に比較的高い放射活性が認められた．比較のために行った雄性ラットへの$^{14}$C-DIP-Pの経口及び静脈内投与，更には$^{14}$C-disopyramide の静脈内投与後のオートラジオグラムから，本実験に用いたdisopyramideは一般に肝臓，腎臓，胃，小腸に局在しやすいことが明らかとなった．また，投与12時間後でも胃及び小腸に高い放射活性を示していることから（Fig. 6, 7, 8），この薬物の胃からの分泌及び胆汁排泄の可能性が推測された．

## 結　論

DIP-Pを妊娠ラットに静脈内投与して，その血中濃度，糞尿中排泄，胎仔移行，更には乳汁移行を検討し，以下の結果を得た．

1．静脈内投与後，放射活性はすみやかに組織に移行又は排泄されて，血中濃度は12時間目の極大を無視すると，2相性のすみやかな減少を示した．

2．糞尿中排泄量は，48時間で80.5％であり，糞中排泄量の方がはるかに多かった．

3．胎仔への放射活性の移行はすみやかであるが，母体血中濃度の急激な低下につれて，すみやかに消失していった．

4．乳汁移行はすみやかであり，かつ母体血中濃度より高いが，血中濃度の急激な低下に比例的にすみやかに減少した．

5．妊娠ラットのオートラジオグラムから，この薬物は胎盤を通過し，胎仔へ移行することが認められたが，その程度は微量であり，かつすみやかに消失した．

## 文　献

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